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The Effects of High Colony Density on Filtration, Mortality and Behavior in the Zebra Mussel, Dreissena Polymorpha

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THE EFFECTS OF HIGH COLONY DENSITY ON FILTRATION, MORTALITY AND BEHAVIOR IN THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA*

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CHAPTER I

LITERATURE REVIEW

The zebra mussel, *Dreissena polymorpha*, is a small fresh water bivalve that originated in Eastern Europe, probably in the Northern Black Sea/Caspian Sea region near the Dnieper and Volga rivers (Ludyanskiy, 1993). The zebra mussel has a history of invading new habitats and quickly dominating the benthos. Documented invasions occurred in England in 1824 (Morton, 1971a), in the Netherlands and the Rhine River in the 1830's (Smit et al., 1993; Neumann et al., 1993), in Northern Poland in the middle 1800's (Stańczykowska & Lewandowski, 1993), and most recently, in the Great Lakes of North America in the mid 1980's (Hebert et al. 1989).

The zebra mussel was first identified in North America off the eastern shore of Lake St. Clair in 1988 (Hebert et al., 1989). Based on the size of the individuals discovered, it was estimated that they first entered the lake in late 1985 or early 1986, and were probably introduced through the released bilge water of an eastern European freighter. Within a few years of their introduction to Lake St. Clair, the zebra mussel quickly spread to all of the five major Great Lakes.

Dreissena are part of the order Veneroida, superorder Eulamelliobranchia. They are distinct from other freshwater bivalves for several

reasons: (1) they possess a byssal apparatus, a gland that secretes proteinaceous strands by which they attach to hard substrates (Eckroat et al., 1993), (2) they are heteromyarian (anterior and posterior adductor muscles of different size) rather than isomyarian (same size adductors), (3) they have a non-parasitic, pelagic, larval or veliger stage, and (4) they gregariously settle and form large, dense, colonial aggregations. With these traits, the zebra mussel is more like many marine bivalves (e.g. Mytilidae) than other freshwater groups.

One of the reasons for the rapid spread of zebra mussels is their tremendous reproductive capability. Large adult females are capable of producing more than 1.6 million oocytes (Neumann et al., 1993) each reproductive event. Male and female zebra mussels synchronously release their gametes into the water column. This spawning is triggered by temperature cues, mediated by serotonin (Ram & Nichols, 1993), and can occur once or twice a year. The larval, or veliger stage of development is pelagic, enabling zebra mussels to quickly inhabit new areas by being carried by prevailing currents. After several weeks, veligers drop from the water column and settle on hard substrates.

Like other bivalves, zebra mussels obtain food by filtering particles out of the water column. Ciliary action creates flow through an incurrent siphon that protrudes beyond the mantle cavity. Water drawn into the siphon passes through the gills, which mechanically filter out suspended particles, and then

passes out of a ventrally located excurrent siphon. Particles trapped in the gills are processed, with selected food items being digested in the gut, and non-palatable items being packaged in mucilaginous pellets called pseudofaeces and expelled out the incurrent siphon. The mechanism by which zebra mussels select food items is not completely known, but is likely a combination of size and chemosensory selection. Sprung and Rose (1988), determined that zebra mussels could effectively filter particles greater than 0.7μm in diameter, and Mikheyev (1967) determined that zebra mussels could ingest particles as large as 450μm in diameter. However, in a study of stomach and mid-gut contents, Ten Winkel and Davids (1982) showed that only particles in the 15 to 40μm size range were actually digested; most of these were diatoms and other algae. Larger particles were rejected, and smaller ones passed through the gills and out the excurrent siphon.

The rate of filtration by zebra mussels, as well as other bivalve species, has been well documented. Morton (1971b) performed a series of experiments on the effects of several factors on filtration, including pH, temperature, fluid viscosity, particle density, and shell length. It was observed that filtration ability peaked at pH 7.0 and increased as temperature increased to 35°C However, the validity of some of the results from these tests is in question. The experiments used colloidal graphite as a 'food' source, which might have inhibited feeding response. Also, the proposed maximum filtration rate was at a temperature of 35°C, which is several degrees higher than the maximum

documented survivable temperature. Walz (1978) and Sprung & Rose (1988) examined the effects of food size and density on filtering ability. These results indicated peak filtration at particle sizes of ~5μm, particle densities of 15 cells/μl, and temperatures of ~12.5°C. Kryger & Riisgard (1988) compared zebra mussel filtration rates with those of several other bivalves and found slightly lower filtration rates in individual zebra mussels as compared with other bivalves, presumably due to their smaller size. Each of these studies used green algae, most often the genus *Chlamydomonas* (~5μm), as a filtrate, with a coulter counter used to assay algal densities.

Because so many characteristics of *D. polymorpha* differ from other freshwater bivalves, the most relevant related organisms are found in marine benthic environments. A sizeable body of work has been done by several investigators on the filtration, behavior, and population dynamics of several marine species, including the blue mussel, *Mytilus edulis*, the ribbed mussel, *Geukensia demissa*, the barnacle, *Semibalanus balanoides*, and several bryozoan species. These organisms are either infaunal or epifaunal, usually have high population densities, and are either passive or active suspension feeders. For each species, competition for food, often a function of space, was a determining factor in colony density and population dynamics.

One important trend in food consumption was the effect of individual location within a colony. Frechétte et al. (1992) reported slower growth rates for *Mytilus edulis* individuals located downstream from other colonies than

upstream individuals. This slow growth was further depressed by an increase in the downstream colony size. Similar results were obtained by Okamura (1992) measuring food uptake by the marine bryozoan *Electra pilosa*, in both lab and field conditions. Pullen & LaBarbera (1991) determined that barnacles located in the upstream portion of natural clusters in unidirectional flow had significantly higher food particle capture rates than barnacles located downstream.

The role of flow speed on particle capture rates and food consumption is not as clear. Wildish and Miyares (1990) concluded that higher flow speeds (>25cm/s) decreased the filtration rate of the blue mussel in a recirculating flume. Wildish et al. (1987) reported similar findings for the giant scallop *Placopecten magellanicus* at high flow speeds (>10-20cm/s). In both cases, these high flow speeds probably interfered with the cilial pumping action of the incurrent siphon, decreasing pumping efficiency. Similarly, Okamura (1992) determined that higher flow speeds (~7cm/s) reduced the colonial feeding rates of the marine bryozoan *Electra pilosa*.

However, higher flow speeds can have the opposite effect. In a study on the freshwater bryozoan *Plumatella repens,* higher flow speeds (5.3cm/s) increased feeding rates (Okamura & Doolan, 1993). These variations in filtering responses to higher flow speed are probably due to the morphological and behavioral differences of the organisms studied. *Plumatella repens* has a larger, differently shaped food gathering lophophore and more tentacles than

E. pilosa (Okamura & Doolan, 1993), and thus displays a different response to higher flow speeds.

For many of these colonial filter feeders, it is likely that the effects of flow rate on individual filtration are determined by their location within the colony. Patterson (1984) showed that in the colonial octocoral *Alcyonium siderium*, downstream polyps have lower filtration rates than upstream polyps in low flow speed conditions (2.5 cm/s), but have higher filtration rates than upstream polyps in high flow speed conditions (19 cm/s). This was probably due to the increase of downstream turbulence by the rough surface of the upstream polyps, leading to greater mixing of food particles in the vicinity of the downstream polyps (Patterson, 1984). A similar trend is seen in large aggregations of the barnacle, *Semibalanus balanoides*. While individuals in the upstream portion of a barnacle aggregation are exposed to fast laminar flow, downstream individuals are exposed to slower, more turbulent flow (Pullen & LaBarbera, 1991), which would increase the feeding rate of individuals there relative to those upstream.

Behavioral changes also affect filtration rates relative to flow speed. A study on the marine bryozoans *Bugula neritina* and *B. stolonifera* indicated that while flow speed had no overall effect on feeding rate, it did alter the average size of captured particles (Okamura, 1990). This change was probably due to an alteration of feeding behavior from the use of ciliary currents to the use of tentacular feeding (Okamura, 1987). Another behavioral response to higher

flow speed has been observed in the barnacle. Trager et al.(1990) showed that in rapidly flowing water (>3.1 cm/s), barnacles passively fed with their cirral nets extended, while barnacles in slower flow conditions (<3.1cm/s) actively swept their cirri into the flow to capture food. This behavioral switch also occurred in the downstream portion of barnacle aggregations, but was due presumably to decreased food availability in more turbulent flow conditions. Because passive filtration is more energy efficient than active filtration, and because of the greater rate of food particle capture in the upstream portion of barnacle aggregations, the individuals there have an advantage over individuals located downstream (Pullen & LaBarbera, 1991).

Another important aspect of flow that determines food availability is the role of the benthic boundary layer. The benthic boundary layer is a gradient of decreasing fluid velocity as the fluid-solid interface of the substrate surface is approached. The thickness of a boundary layer is defined as the distance from the bottom surface required to achieve a velocity which is 99% of that of the free-stream value (Vogel, 1981). The benthic boundary layer affects food availability for benthic filter feeders in several ways. By reducing flow speeds, it helps dense colonies of filter feeders deplete the surrounding water of food (Wildish & Kristmanson, 1984). As food is depleted by filter feeders near the colony surface, the benthic boundary layer impedes the recirculation of food from the overlying water. With the reduction in local flow speed, food depleted water is maintained at the colony surface, and a concentration gradient is

formed (Wildish & Kristmanson, 1979). The end result is that although overlying food concentrations may be quite high, that food is unavailable to filter feeders within the benthic boundary layer.

Fréchette & Bourget (1985a) observed zones of food-depleted water up to 5cm above intertidal mussel beds in the St. Lawrence Estuary. Similar *in situ* areas of depletion were found by Muschenheim & Newell (1992) over large blue mussel beds, indicating that their effective filtration range was only 3.5cm above the colony. In flume studies with model bivalves, O'Riordan et al. (1993) showed that food availability increased with height above the sediment within a stable benthic boundary layer. In flume experiments with the blue mussel and the horse mussel, *Modiolus modiolus*, Wildish & Kristmanson (1984) found that food concentrations at various heights within a stable benthic boundary layer were equivalent at ambient flows of ~4cm/s, and statistically lower than food concentrations above it. When blue mussels were raised 1.0M above a mussel bed, their growth rates increased (Fréchette & Bourget, 1985b).

The two most important factors governing the establishment of a stable benthic boundary layer are surface roughness and ambient flow velocity. Greater surface roughness increases turbulence, which inhibits the establishment of a stable benthic boundary layer. Greater ambient flow velocity reduces the thickness of the benthic boundary layer, increasing flow speeds nearer the fluid-sediment interface (Muschenheim et al., 1986). It also increases the turbulent effects of rough sediment surfaces (Fréchette et al.,

1989). In large scale flume experiments, Butman et al. (1994) demonstrated that the overall consumption rates of blue mussels are dependent on ambient flow speed in the presence of a stable benthic boundary layer.

The large-scale disturbance of natural turbulence (e.g. tidal action) can often disrupt, or even invert, vertical food concentration gradients (Fréchette & Bourget, 1985a). Turbulent mixing caused by the surface roughness of the sediment, including the filter feeders themselves, or by the flows generated by feeding currents, will also reduce the effects of the concentration boundary layer, bringing water with higher food concentrations to the bottom (Fréchette et al., 1989). As such, the presence and the activities of benthic filter feeders themselves can heavily impact a concentration regime that is dominated by physical fluid forces. In many cases vertical food gradients could impact consumption rates more than horizontal gradients.

In contrast to the wide ranging studies on marine benthic filter feeders, little is known about the interactions of lentic freshwater benthic filter feeders, mainly because there are few similar species to zebra mussels. Larval blackflies, *Simuliidae*, are active filter feeders and often aggregate in large groups, often in excess of 600,000 individuals/m^2 (Wotton, 1992). Found predominantly in shallow, fast flowing streams, larval blackflies show many behavioral responses to flow, including changes in feeding posture (Hart et al., 1991), and in labral fan structure (Lacoursière & Craig, 1991) in response to varying flow rates. They also exhibit fierce territoriality, engaging in aggressive

behavior in order to displace upstream neighbors; this was presumably to increase food availability, as aggressive behavior decreased when food was more available (Hart, 1987).

Another relevant freshwater community is the algal biomat. Like zebra mussels, algal communities have vertical, as well as horizontal, community structures. Algal communities have been demonstrated to experience strong vertical gradients of both light (Jorgensen et al., 1983) and nutrients (Burkholder et al., 1990), both of which diminish with depth in the mat. In response to these gradients, some diatoms were observed to migrate vertically (Wasmund, 1984), away from areas of poor quality.

The synthesis of these studies reveals a complex, dynamic interdependence of multiple factors on the filtration rates of individuals within colonies. The speed and pattern of ambient flow, the density of available food particles, the size, shape, and density of the filtering colonies, the location of individuals within those colonies, and physical forces like the benthic boundary layer are all factors that stimulate the competition for food. These factors determine relative food availability to individuals in different locations in groups of benthic suspension feeders; they should affect not only individuals, but colonial aggregations as a whole. Presumably, the size and shape of colonies could be predicted based on local flow conditions, and could be expected to remain constant over time, provided that flow conditions and food resources remain constant (Pullen & LaBarbera, 1991).

CHAPTER II

INTRODUCTION

Most research on zebra mussel ecology has focused on the large scale impacts that entire populations have on the local aquatic ecosystem. These impacts have been predicted to be severe, due to the high population densities that zebra mussels can attain. By filtering the water for food particles, dense zebra mussel populations have been predicted to be capable of removing large amounts of phytoplankton from the water column, as much as 96% in some areas (MacIsaac et al., 1992). Phytoplankton removal has several major large-scale effects, including a significant increase in water clarity and light penetration (Smit et al., 1993), the subsequent increase in benthic algal (Pillsbury & Lowe, 1994) and invertebrate (Tuchman & Bradford, unpublished data) biomass, and the disturbance of the pelagic food web, affecting many invertebrate and fish species.

Studies of basic zebra mussel biology have usually been performed in a laboratory setting, and have involved either individual or small groups of zebra mussels. In nature, however, zebra mussels most commonly exist as large, dense colonies. Presently, the only studies on the colonial aspects of zebra mussels have involved post-veligers, indicating that they preferentially settle

among previously established adult zebra mussels rather than on uninhabited substrates (Lewandowski, 1976).

Current estimates of individual zebra mussel filtration rates, and the predicted rates of filtration of entire zebra mussel populations do not reflect *in situ* conditions. These predictions are primarily based on studies of zebra mussel filtration that involve individuals or small groups of mussels, and that use small volumes of water that is either uncirculated, or in undefined flow conditions. As yet, no experimentation has been conducted using *in situ* densities of zebra mussels with controlled flow conditions.

The microenvironmental effects of zebra mussel filtration, including the effects that individual zebra mussels have on each other, are also poorly understood. Zebra mussel colonies have been known to achieve thicknesses greater than 15cm on artificial substrates (e.g. intake pipes), and at least 5cm on natural substrates (pers. observ.). Interactions between individuals, and the conditions in different locations within these dense colonies, have not been studied. The effects of filtration, respiration and excretion on the interstitial water within these colonies, and the subsequent effect of these conditions on zebra mussel behavior and mortality warrant examination.

Several aspects of zebra mussel motility also warrant examination. It has been observed that smaller mussels tend to be more mobile than larger ones, and that there is a tendency for many mussels, especially smaller ones, to move up to the water's edge in captivity (Marsden,

pers. comm.). Mussels are capable of movement in temperatures as low 4°C, and can achieve migratory speeds of 53cm/hour (Cawein, 1993). They have also been observed to exhibit searching behavior, and are capable of mass migrations, possibly due to sudden changes in surrounding conditions. As yet, no other work has been done to examine movement, or density-dependent effects on individual mussel movement.

By studying the role of group living in zebra mussel ecology, a better understanding of the processes that control zebra mussel populations can be gained, as well as a better understanding of their basic biology, and their effects on local ecosystems.

The overall purpose of this study was to examine the dynamic processes that occur within large, dense zebra mussel colonies, and to examine the role that spatial position within a colony plays in determining the conditions in which individual zebra mussels live.

The objectives of the study were to combine laboratory experiments with several *In situ* surveys of natural zebra mussel populations to determine the effects of high densities of zebra mussels on differences in: (1) interstitial water quality in various locations within a colony, (2) food availability to individuals in various locations of a colony under different flow conditions, and (3) the vertical migration and mortality of individuals in various locations of a colony.

CHAPTER III

MATERIALS AND METHODS

The Effects of High Zebra Mussel Density on Interstitial Water Quality

In order to determine the effects of high zebra mussel densities on interstitial water quality, water samples were extracted from several locations within reconstructed dense colonies. Because zebra mussels excrete waste in the form of ammonia (NH_4-N), which is quickly oxidized into nitrate (NO_3-N) in the presence of dissolved oxygen, the two forms of dissolved inorganic nitrogen were used as indicators of nitrogenous waste.

Zebra mussels were collected using SCUBA at several sites along the southwestern shore of Lake Michigan. After collection, mussels were maintained for at least one week in a 1100 gallon flume of unfiltered Lake Michigan water at the Lake Michigan Biological Station of the Illinois Natural History Survey in Zion, Illinois.

Flow rate in the flume was measured by injecting dye into the flume at the location of upstream chamber placement. The time necessary for the leading edge of the dye plume to travel 100cm was recorded, and flow was maintained at an average velocity of 1cm/s.

Six zebra mussel colony chambers were constructed using unglazed

ceramic tiles as a base, and 1.5mm mesh aluminum screening for the sides. Aquarium grade silicon sealant was used to affix the screening onto each tile, forming a cylindrical chamber 7cm high and 20cm in diameter. The silicon was allowed to dry for at least 24h prior to use.

Six colony chambers were filled to a height of 6cm with live, haphazardly sized zebra mussels. Each colony chamber was rinsed with lake water to remove debris and ensure initial homogeneity of interstitial water, and placed into one of the 1100 gallon flumes of unfiltered Lake Michigan water at the Lake Michigan Biological Station (Fig. 1). The chambers were placed on cinder blocks to elevate them from the bottom, which put them in an area of faster, more laminar flow, and exposed them to ambient conditions within the flume.

After 4hrs., 20ml samples were taken with a sterile 5cm long stainless steel needle and a 60ml syringe. Each needle was carefully inserted, with each sample drawn slowly to minimize the disturbance to local interstitial water. In some cases, smaller samples of water were taken from multiple points in the same location to draw the required 20ml of sample. Samples were taken from three horizontal (Upstream, Midstream, and Downstream) and three vertical (Top, Center, Bottom) sites (Fig. 2), for a total of nine interstitial sites, plus an ambient site upstream of the colonies.

After removal, each sample was immediately filtered through acid washed 0.45μm cellulose filter into a scintillation vial. To stabilize each sample,

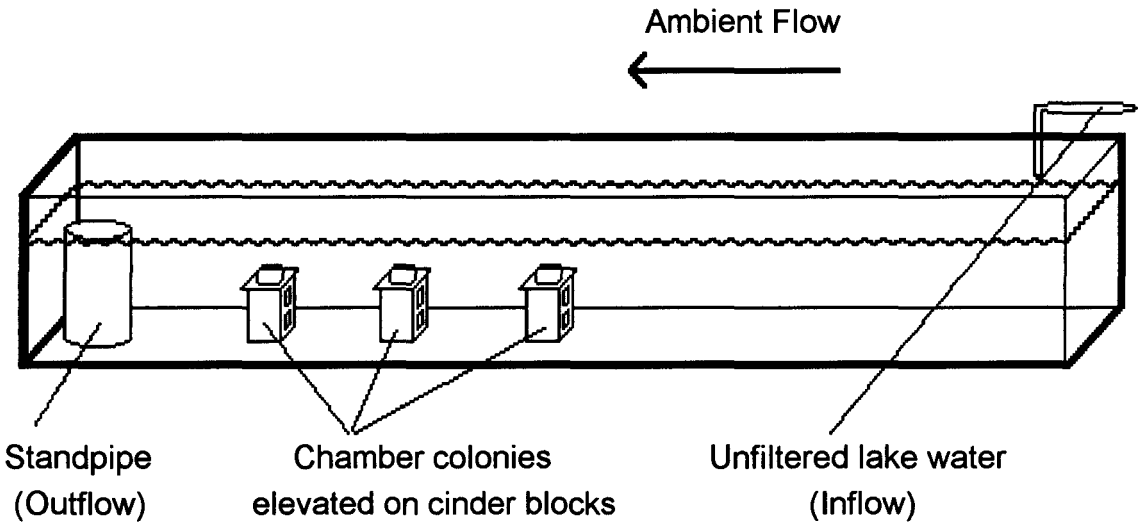

Figure 1. Unidirectional flow-through flume at the Lake Michigan Biological Station, Illinois Natural History Survey, Zion, IL. Dimensions: 32' Length x 31" Height x 36" Width. Water depth = 24.25". Flow speed = 1cm/s.

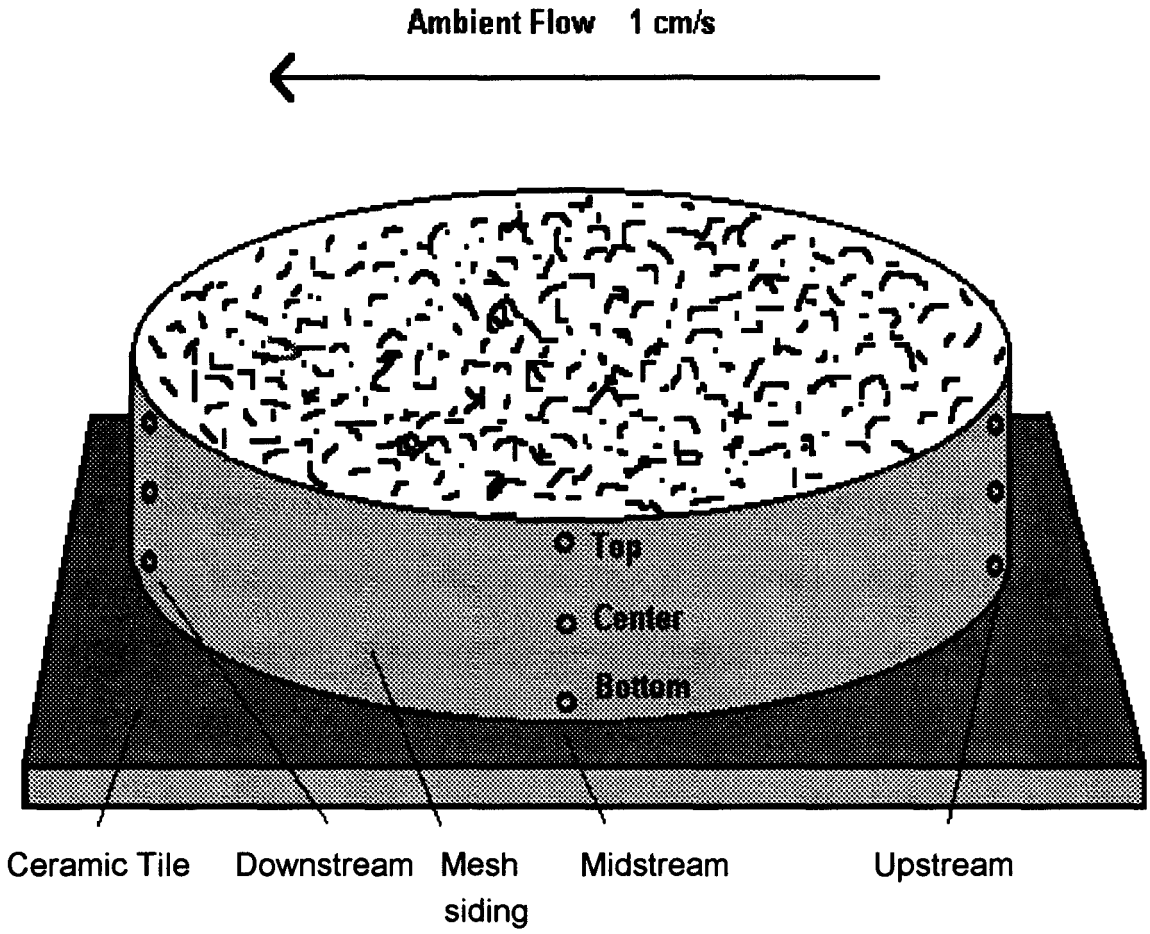

Figure 2. Side view of zebra mussel colony chamber with nine interstitial sampling sites. Colony dimensions: 20cmdiameter, 6cm height. Ambient samples were taken upstream from the colonies.

sulfuric acid was added to bring it to ~pH 2.2. Each vial was capped, labelled and placed in ice. Each sample was analyzed with a Technicon II Auto-Analyzer for NH_4-N and NO_3-N content using dissolved NH_4-N and NO_3-N sampling methods (APHA, 1989).

Interstitial dissolved oxygen (D.O.) levels were taken with a Y.S.I. electronic oxygen meter and probe at the same eleven sites immediately after the water samples were taken. The probe was slowly inserted into each site within the colony to minimize any disturbance of the local interstitial water.

In Situ Survey of Interstitial Water Quality and Size Class Distribution

In order to determine the effects of naturally occurring conditions and zebra mussel densities on *in situ* interstitial water quality, and to determine the vertical stratification of size classes, a SCUBA survey was conducted at 4-6m depths along a stone breakwater at the Port of Indiana in southern Lake Michigan. The site was chosen because it was known to have high zebra mussel densities.

Interstitial water was sampled at the site with plastic 60ml syringes and sterile 5cm needles. A syringe was placed with the needle tip at the base of the colony, and 20ml of interstitial water was slowly removed. A syringe was then placed at the colony-water column interface at the same location, and a second 20ml of water was collected. Another syringe was used to sample 20ml of ambient water approximately 2m from the colony surface. After sampling,

each needle was capped to prevent leakage or the introduction of lake water. One pair of samples was collected from each of ten colony sites. On shore, each water sample was processed and analyzed for NO_3-N and NH_4-N concentrations as in above.

Eight aluminum coring devices with an 8.6cm diameter and either 5cm or 4cm depth were used to sample zebra mussel colonies. Sampling sites were selected based on high zebra mussel densities and a relatively flat area of rock substratum; these tended to be on vertical rock faces. At each coring site, a ruler was inserted into the zebra mussel colony to determine thickness. The coring devices were firmly pushed into the zebra mussel colony until they reached the substratum. A thin steel plate was then slid under the core to dislodge mussels from the substrate. Pre-cut disks of porous foam padding were inserted on top of the surface of the colony to fill any remaining space in the core, and prevent the dislodging of individual mussels. Each end of the core was capped with a plastic lid, and the complete core was placed in a dive bag.

On shore, each core was immediately hand separated into two layers: the bottom 2cm, and the remaining upper layer, which varied in thickness. These layers were then rinsed in lake water to remove debris, placed into glass containers and labelled. Each container was filled with lake water to maintain mussel viability, and transported to Loyola University Chicago. The mussels in each container were separated into four different size classes: (I)

<6mm, (II) 6-13mm, (III) 13-20mm, (IV) >20mm, and further divided into groups of live or dead individuals.

The Effects of High Density and Variable Flow Rates on Filtration Ability

To determine the effects of varying ambient flow and varying individual positions on filter feeding in dense zebra mussel colonies, filtration experiments were performed in the laboratory in four small recirculating flumes.

Four vertically recirculating flumes were constructed of glass and silicon based on a design from Vogel & LaBarbera (1978). Each flume was designed to minimize volume and maximize control of flow rate (Fig. 3). Plastic columnators and baffles were inserted into each flume to attempt to establish laminar flow through the experimental section of the flume.

Zebra mussels were collected from several sites in southwestern Lake Michigan using SCUBA, and placed in holding tanks. The mussels were slowly acclimated to experimental physical and chemical conditions and starved for at least 24h prior to each experiment. A species of *Chlamydomonas* was cultured in a 7L container with Alga-Grow® and a phosphorous-free Guillard's medium, to be used as a food source for the zebra mussels during the experiments. Six days before the experiments began, the culture was inoculated with 4mCi of ^{32}P as PO_4. The inoculated culture was filtered and spun down to remove unmetabolized ^{32}P, and resuspended in water with Guillard's medium.

The algal density of the stock culture was determined by averaging 6

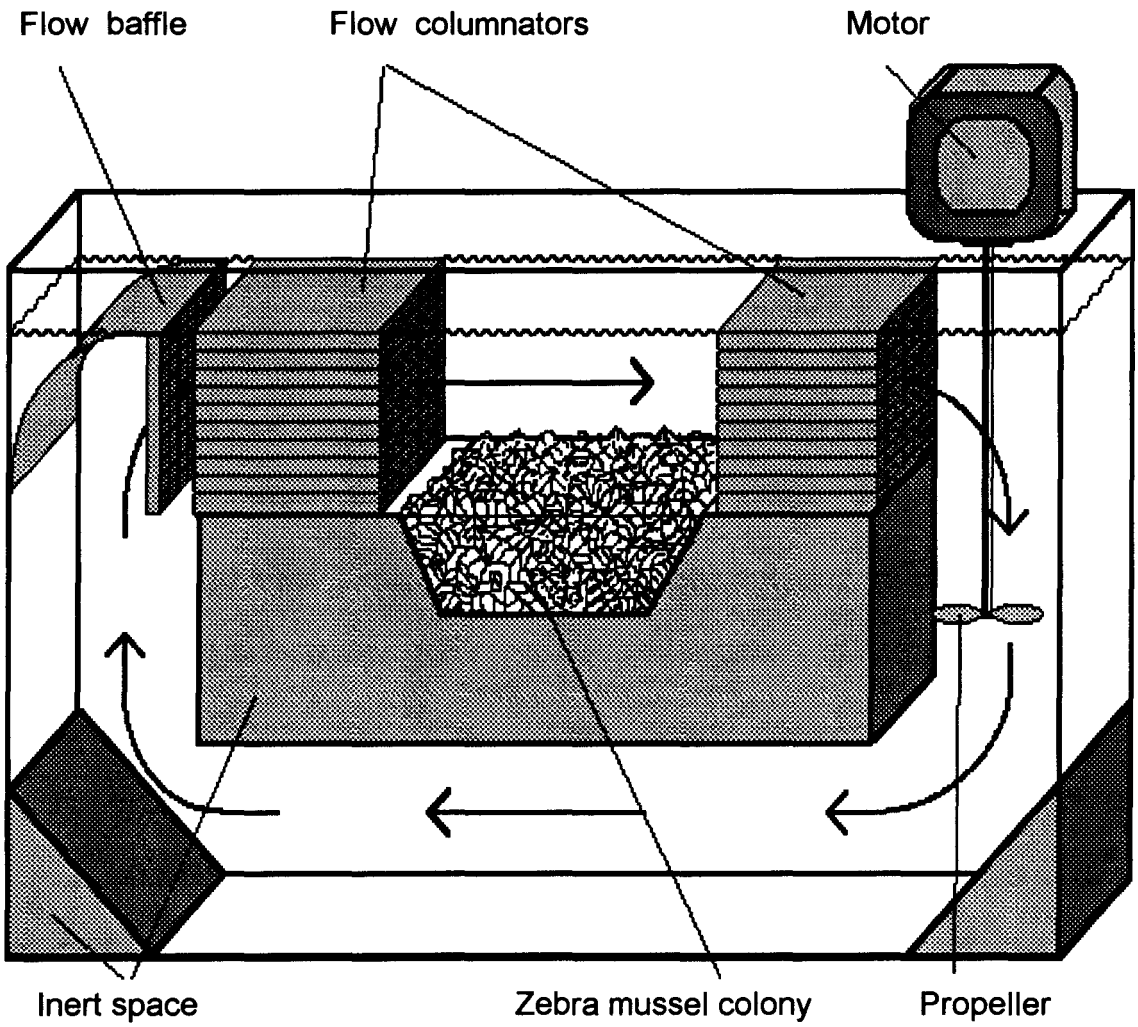

Figure 3. Recirculating glass flume used in colony filtration experiments. Working volume = 10L. Internal dimensions: 50cm length x 30cm height x 10cm width.

cell counts with a hemacytometer. The radioactivity of the stock culture was measured in a Beckman LS 7000 scintillation counter and compared to the algal density to estimate a cpm/cell level in the culture.

Each feeding experiment was conducted in the four recirculating flumes, with motors used to generate flow rates of 2.5, 10, and 20cm/s. Airstones were added to the control flume to produce turbulent mixing and simulate the undefined flow conditions used in previous experiments, with a net flow velocity of 0cm/s. Flow rate was determined by timing the rate at which small neutrally buoyant particles travelled across a measured 10cm length along the working area of the flume. Enough live zebra mussels from the holding tanks were distributed into four acrylic chambers (9cm x 10cm) to fill them to a height of 6cm. They were then placed into each flume, and allowed to acclimate to conditions for at least 30min prior to each experiment, and observed to ensure normal filtering activity was occurring. Seventy milliliters of homogeneous algal culture was added to each flume, and the zebra mussels were allowed to filter for 30 minutes. Three replicates were conducted for each ambient flow speed.

After filtration, the colony chamber was removed from the flume and drained of water. The zebra mussel colony was then separated into three 2cm horizontal layers. Zebra mussels in the top and bottom layers were further divided into one of three size classes: (I) <10mm, (II) 10-20mm, and (III) >20mm shell length. A random sub-sample from each layer and size class was weighed, placed into a blender, and homogenized in 50ml of distilled water for

two minutes. This mixture was filtered through several layers of cheese cloth to remove shell particles and other large debris. Three 1ml samples of filtrate were taken, and each was added to 4ml of a 10% trichloroacetic acid (TCA) solution in a test tube and refrigerated for 24hrs to precipitate any dissolved radioactive matter. To remove the radioactive matter from each sample, they were vacuum filtered through glass fiber filter paper and rinsed with a 10% TCA solution. Because TCA residue can quench radioactive signal, the filters were rinsed with a 95%ethanol solution to remove any remaining TCA, and allowed to dry for at least 10min. After drying, each filter was placed face up in a scintillation vial, to which 10ml of Aquasol scintillant was added. Each vial was measured for radioactivity in a Beckman LS 7000 scintillation counter.

The Effects of High Zebra Mussel Density on Migration and Mortality

In order to examine the effects of vertical water quality gradients on mortality and migration rates, individual zebra mussels were tracked in several chamber colonies for different lengths of time. Zebra mussels for this experiment were collected using SCUBA at several sites in southwestern Lake Michigan and transported to the Lake Michigan Biological Station. There they were placed in the 1100 gallon flume with unfiltered Lake Michigan water and allowed to acclimate to flume conditions for at least one week.

Several thousand mussels were labelled with paint in order to track their vertical movements within the chamber colonies in each of the experiments.

Randomly selected groups of mussels, consisting of all size classes, were removed from the flume, washed in Lake Michigan water to remove debris, divided into three groups, and allowed to air dry for one hour. Each of the three groups was uniformly sprayed with a different color of acrylic paint. To insure that no paint came in contact with the soft tissues of the mussels, they were agitated to induce shell closure before spraying. The mussels were allowed to dry for one hour and then placed in buckets to keep the groups separate. Each bucket was sealed with nylon mesh screen and reintroduced to the flume.

Previous observations had shown that zebra mussels can tolerate extended periods out of the water (McMahon et al., 1993), however, to ensure that the procedure had not unduly stressed them, the mussels in each bucket were examined one day after spraying. Mussels with gaping shell openings that did not close with mechanical stimulation or that lacked soft tissues were considered dead or dying and were removed.

Colony chambers used in the experiments were identical to those mentioned above. To simulate a large, dense colony, haphazardly selected individuals from the three color groups were placed in three separate horizontal layers within the colony chambers. Layers of unpainted mussels, which were not tracked, were placed in between the three painted layers to keep them separate and distinct. Several hundred haphazardly chosen painted individuals were placed onto the bottom of each of the chambers. These painted mussels were covered with haphazardly selected non-painted zebra mussels to a height

of at least 2cm from the bottom of the colony chamber. The chambers were then filled with another color of haphazardly selected painted mussels, followed by non-painted mussels to a height of at least 4cm from the bottom of the colony chamber. The chamber was topped off with the last group of painted mussels and non-painted mussels to a height of 6cm. This left each chamber with three distinct horizontal layers of differently colored painted mussels, one layer at the bottom of the colony chamber, one layer between 2-4cm from the bottom, and another layer at the top, roughly 6cm high, with each layer separated from the others by non-painted mussels (Fig. 4).

After each chamber was filled, a nylon screen was placed over the top and attached with a rubber band to prevent mussels from migrating out of the colony during the experiment. The chambers were inserted into the flume and observed in order to ensure minimal displacement of mussels due to the influx of water. Each chamber was placed on a concrete block to elevate it into an area of greater ambient flow (~1cm/s), as in experiment I.

To determine the accuracy of the placement and sampling methods used in the experiments, three chambers were removed immediately after insertion into the water as a control. The experimental chambers were allowed to remain in the flume for periods of 1, 7, 30, or 90 days.

After each trial, the intact chambers were removed from the flume and allowed to drain. To separate the three layers of mussels, horizontal cuts were made in the aluminum screen siding along planes 4cm and 2cm from the

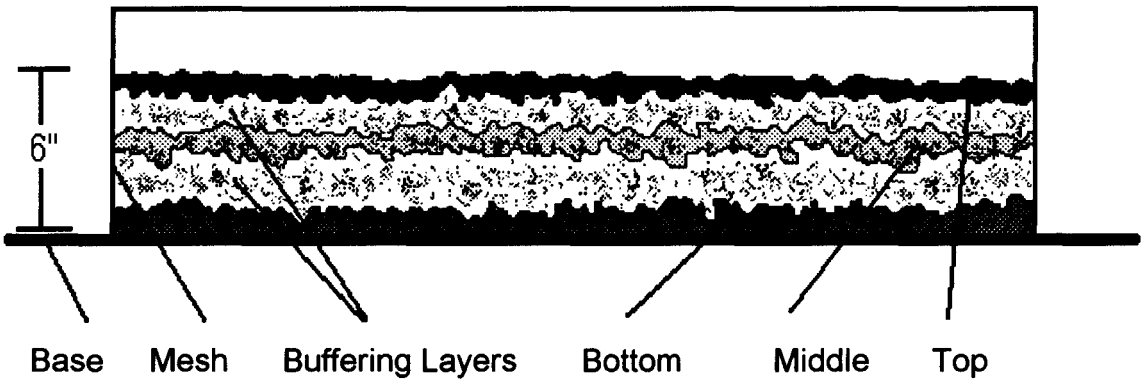

Figure 4. Colony chambers used in migration and mortality experiments. Materials and dimensions identical to those stated above (Fig. 3).

bottom of the chamber. The siding was removed and a wide metal paint scraper was used to remove mussels above each cut horizontal plane. Mussels that were attached with byssal threads to individuals below the plane of separation had their byssal connections severed. This procedure was done carefully to ensure that the zebra mussels were not damaged.

All mussels removed from each horizontal layer were placed into three labelled containers. In instances when an individual zebra mussel bisected a horizontal plane between layers, the mussel was placed with the layer in which the majority of the zebra mussel was located. The zebra mussels in each container were thoroughly rinsed in lake water to remove debris. The buckets were then filled with fresh unfiltered lake water, and airstones were introduced to maintain zebra mussel viability.

The colored zebra mussels in each bucket were separated from the non-colored mussels and placed in large enamel pans. They were then further separated by color and size classes: (I) <6mm, (II) 6-13mm, (III)13-20mm, and (IV) >20mm. Each individual was determined to be alive or dead using the mechanical disturbance method mentioned above. In cases where only single valves were found, an effort was made to match the valve with its opposite. If no opposite was found, the valve was measured and counted as a dead individual.

Three replicates of each time trial were performed, except for the 90 day trial, which was run once, and the 1-day trial, which was run twice.

CHAPTER IV

RESULTS

The Effects of High Zebra Mussel Density on Interstitial Water Quality

In the experiments, DIN levels were usually higher in the interstitial waters of the colonies than in ambient water. Most of this was in the form of NO_3-N, accounting for an average of 85% of total DIN (Appendix Table A). A 3-factor ANOVA was performed on each of the four water quality parameters (NO_3-N, NH_4-N, DIN, D.O.) with vertical position, horizontal position, and colony as the factors (Table 1, 2). Concentrations of NO_3-N increased significantly with depth into the colonies, to 0.835mg/L. NO_3-N also had significantly different concentrations horizontally, with midstream concentrations greater than those upstream or downstream (Fig. 5). Concentrations of NH_4-N were much more variable than NO_3-N levels, which was probably due to the instability of NH_4-N in the presence of oxygen, and there was no significant difference in concentration in the sampling sites within the colony (Fig. 6). Concentrations of total DIN were only significantly different for vertical position, with those at the top being greater than those at the bottom.

Ambient D.O. concentrations were saturated (10.6 mg/L) at 11.4^{o}C temperatures. There was an interaction between vertical and horizontal position

Table 1. 3-factor ANOVA of water quality parameters in low flow (1cm/s)
Fixed factors: Vertical Position, Horizontal Position
Random factor: Colony

Dependent Variable=Ammonia Concentration (mg/L)

Source	df	MS	F	p
Vertical position	2	0.00606	1.006	>0.50
Horizontal position	2	0.00398	0.573	>0.50
Vertical x Horizontal	4	0.00765	0.837	0.527
Colony	3	0.06293	6.885	0.006
Vertical x Colony	6	0.00603	0.660	0.683
Horizontal X Colony	6	0.00694	0.760	0.615
Error	12	0.00914		

Dependent Variable=Nitrate concentration (mg/L)

Source	df	MS	F	p
Vertical position[a]	2	0.13627	35.833	<0.00005
Horizontal position[b]	2	0.03045	9.285	0.025
Vertical x Horizontal	4	0.00501	1.570	0.245
Colony	3	0.01978	6.204	0.009
Vertical x Colony	6	0.00380	1.193	0.373
Horizontal x Colony	6	0.00328	1.029	0.453
Error	12	0.00319		

[a] Post-hoc Tukey test of Vertical Position

Top (0.406mg/L) < Center (0.549mg/L) < Bottom (0.619mg/L)

($p<0.05$) ($p<0.005$)

Top < Bottom

($p<0.001$)

[b] Post-hoc Tukey test of Horizontal Position

Upstream (0.477mg/L) < Midstream (0.596mg/L) > Downstream (0.501mg/L)

($p<0.01$) ($p<0.025$)

Upstream=Downstream

($p>0.05$)

Table 2. 3-factor ANOVA of water quality parameters in low flow (1cm/s)
Fixed factors: Vertical Position, Horizontal Position
Random factor: Colony

Dependent Variable=Total DIN Concentration (mg/L)

Source	df	MS	F	p
Vertical position[a]	2	0.12476	11.016	<0.01
Horizontal position	2	0.04398	3.124	>0.10
Vertical x Horizontal	4	0.02092	1.440	0.280
Colony	3	0.08377	5.765	0.011
Vertical x Colony	6	0.01133	0.779	0.602
Horizontal x Colony	6	0.01408	0.969	0.486
Error	12	0.01453		

[a] Post-hoc Tukey test of Vertical Position

Top (0.513) = Center (0.613) = Bottom (0.715)
(p>0.05) (p>0.05)
Top > Bottom
(p<0.01)

Dependent Variable=Dissolved Oxygen Concentration (mg/L)

Source	df	MS	F	p
Vertical position	2	9.976	49.632	<0.025
Horizontal position	2	0.779	14.981	<0.10
Vertical x Horizontal[a]	4	0.367	6.809	0.001
Colony	5	0.362	6.699	0.001
Vertical x Colony	10	0.201	3.716	0.006
Horizontal x Colony	10	0.052	0.963	0.502
Error	20	0.054		

[a] Post-hoc Tukey test of Vertical x Horizontal Position

TU	TM	TD	CU	CM	CD	BU	BD	BM
10.70	10.68	10.67	10.13	9.88	9.83	9.68	9.17	8.73
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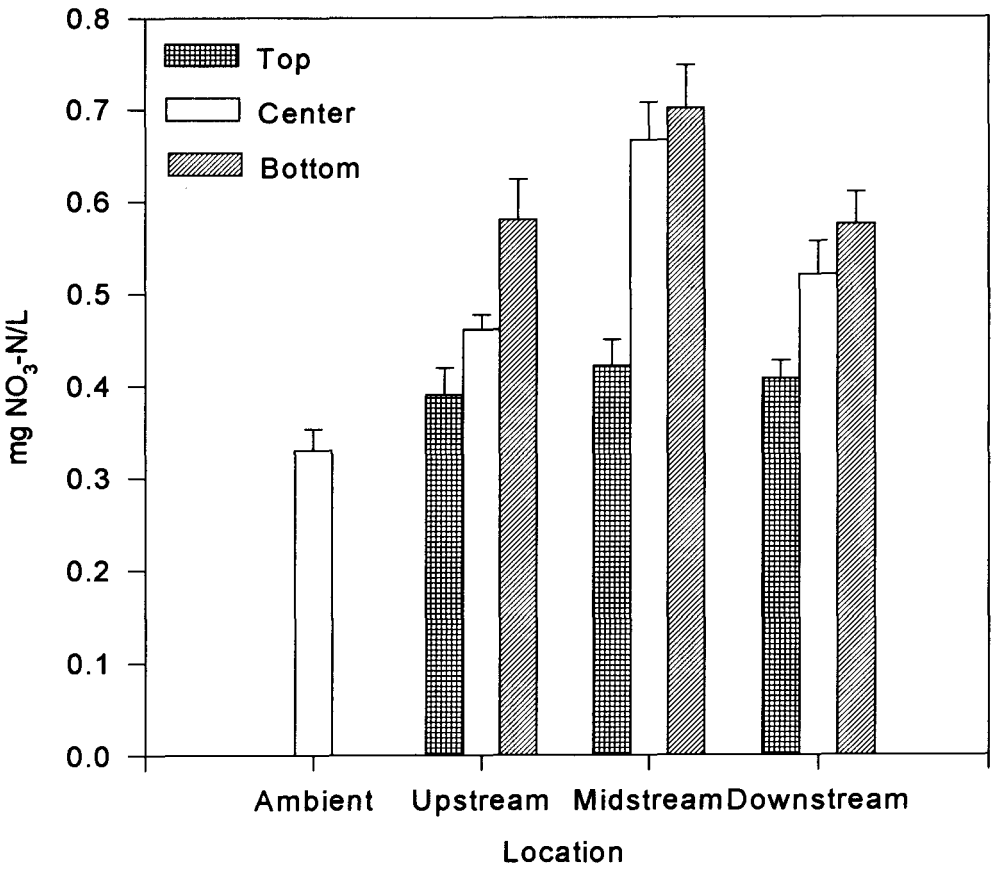

Figure 5. Average concentrations of dissolved NO_3-N at the one ambient and nine interstitial sampling sites in the chamber colonies in 1cm/s flow. Bars represent the mean of 4 replicates + 1s.e.

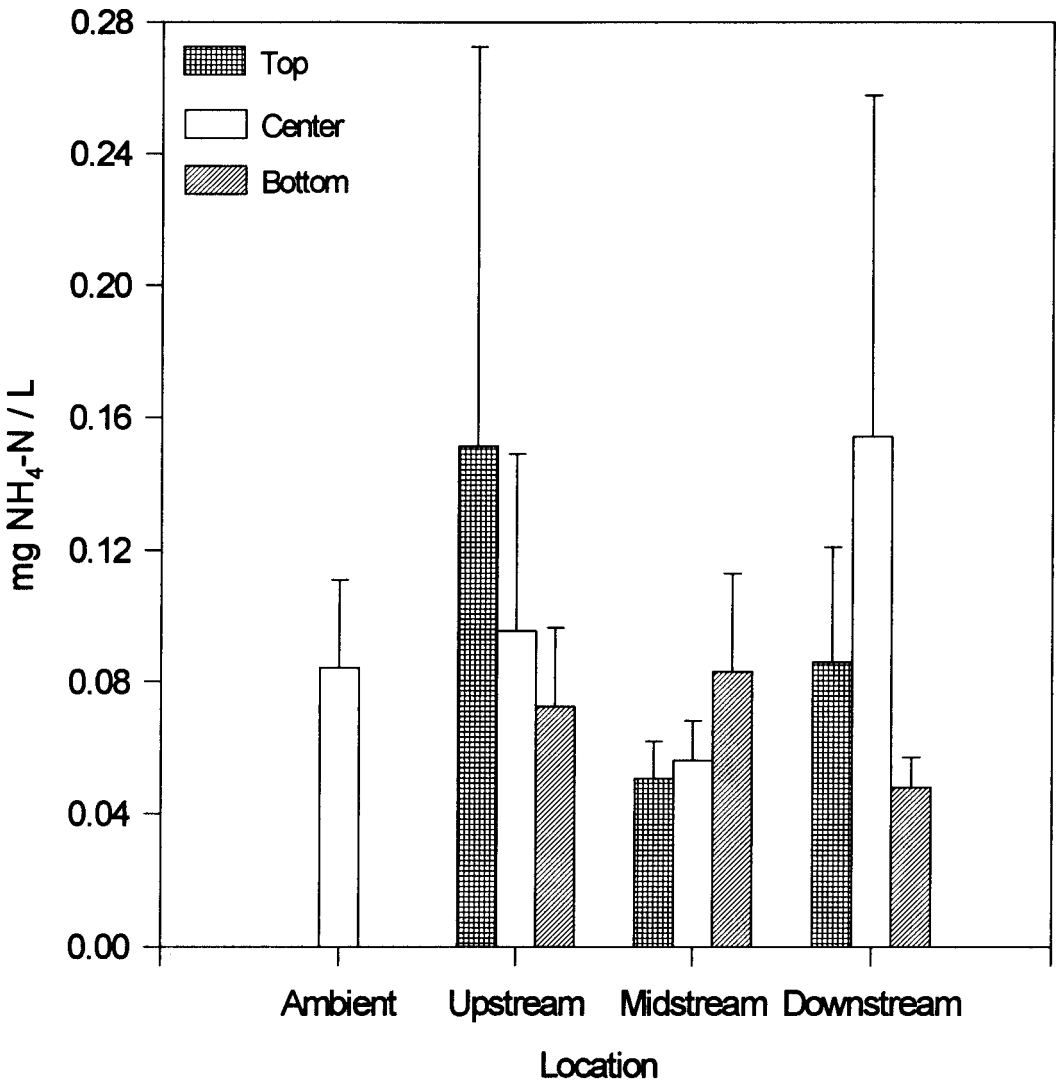

Figure 6. Average concentrations of dissolved NH_4-N at the one ambient and nine interstitial sampling sites in the chamber colonies in 1cm/s flow. Bars represent the mean of 4 replicates + 1s.e.

within the colonies. Horizontally, differences between D.O. concentrations in the top layer sites were not significant. D.O. concentrations in the center and bottom of the colonies were significantly different, with the greatest concentrations being downstream in the center and midstream in the bottom (Fig. 7). In no cases were concentrations of D.O. low enough to establish biologically anoxic conditions; the minimum bottom concentration was 8.2mg/l.

In Situ Survey of Interstitial Water Quality and Size Class Distribution

The zebra mussel colonies at the Port of Indiana site had variable densities. All cores and interstitial water samples were taken on the vertical rather than on the horizontal rock facings because population densities there tended to be much higher; colony thickness ranged from 2cm-5.5cm on the vertical facing, while the colonies on the tops of the rocks were generally <2cm thick.

There was a great deal of variability in zebra mussel population density among the core samples taken (Appendix Table B; Fig. 8), with a mean population density of 41,147/m^2 $\pm$13,340. The size class distribution of mussels was different for the top and bottom layers as well. A 3-Factor ANOVA was conducted on the number of individuals from each size class in the layers, with size class and location (top layer/bottom layer) as fixed variables and core# and colony wet weight as random variables (Table 3). There was a significant interaction between size and layer determining the number of zebra mussels

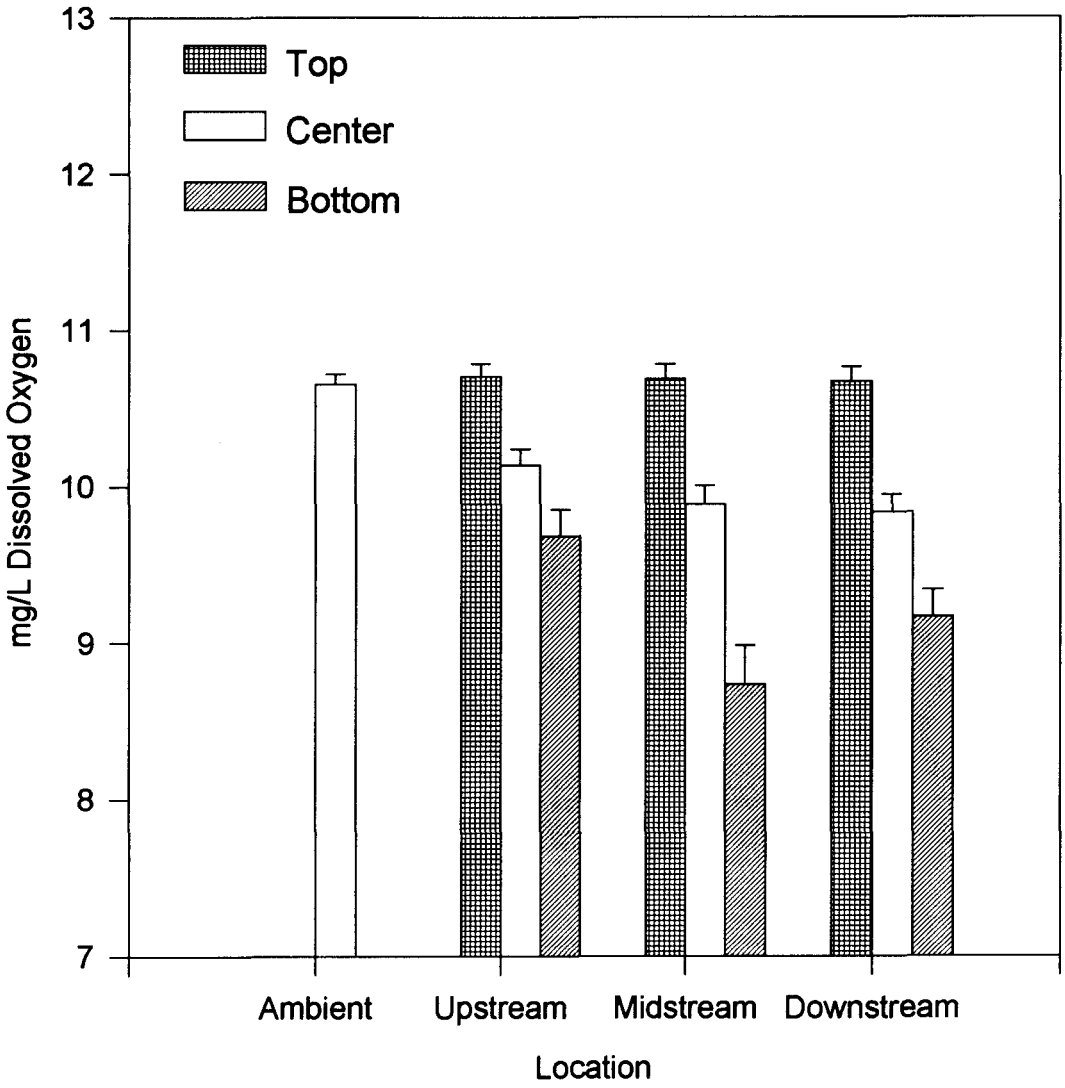

Figure 7. Average concentrations of D.O. at ambient and nine interstitial sampling sites in the chamber colonies in 1cm/s flow. Bars represent the mean of 6 replicates + 1s.e.

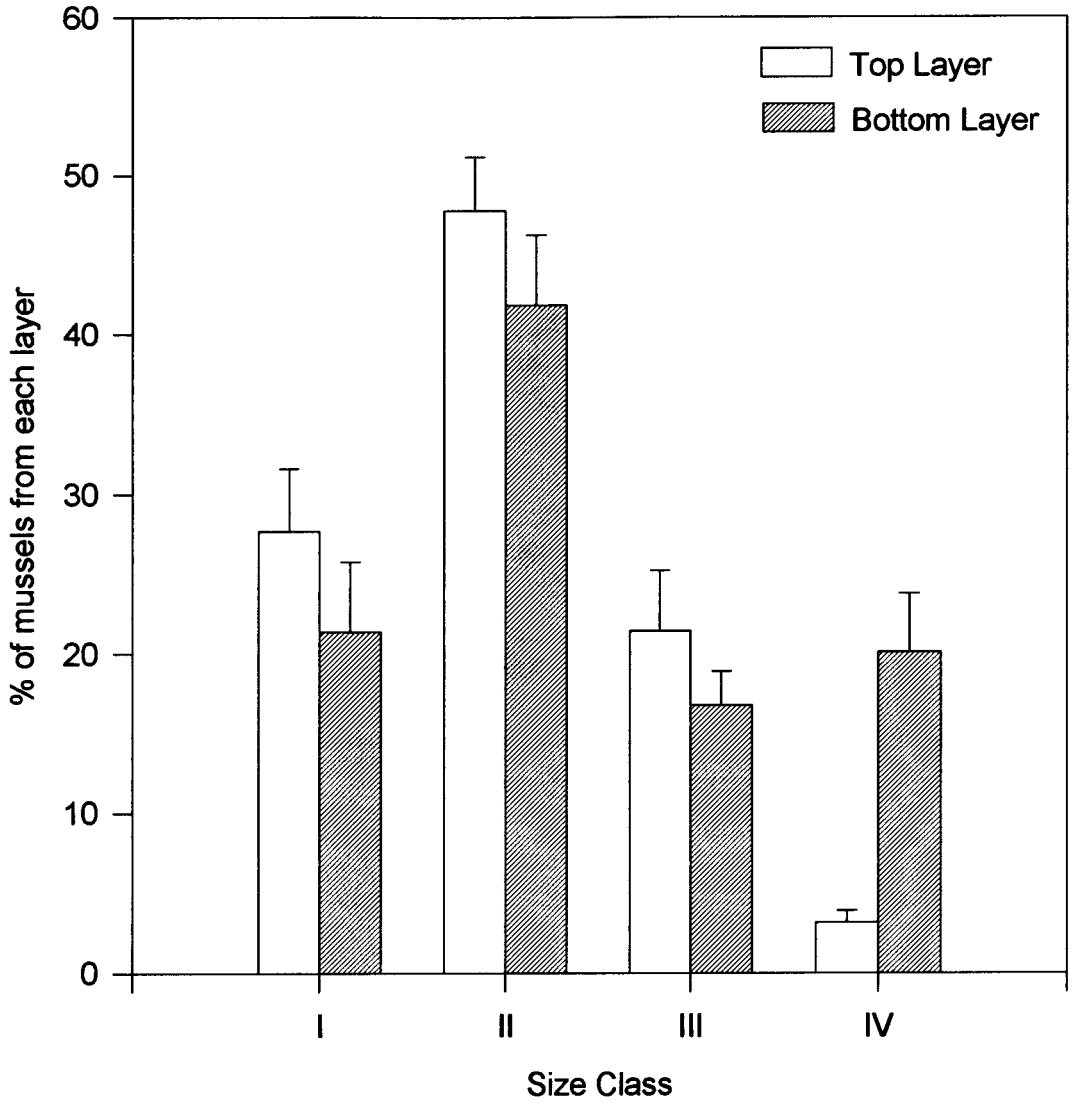

Figure 8. The distribution of size classes in the top and bottom layers of natural zebra mussel colony cores taken at the Port of Indiana, Lake Michigan. Bars represent the mean percentage of total mussels in the layer from each size class + 1s.e.

Table 3. 3-factor ANOVA of relative Size Class Density for each horizontal layer of the cored zebra mussel populations
Fixed factors: Size Class, Position(top/bottom layer)
Random factor: Core#

Dependent Variable=Relative density of mussels

Source	df	MS	F	p
Position	1	8.271	39.853	<0.001
Size Class	3	4.992	24.051	<0.001
Core	7	0.266	1.282	0.306
Position x Size Class[a]	3	2.037	9.814	<0.001
Position x Core	7	0.445	2.143	0.083
Size Class x Core	21	0.250	1.204	0.337
Error	21	0.208		

[a] Post-hoc Tukey test of Position x Size Class

TII	TI	TIII	BII	BI	BIV	BIII	TIV
(2.30)	(1.42)	(0.92)	(0.81)	(0.42)	(0.35)	(0.32)	(0.13)
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found, with differences between top and bottom layers for size classes I and II, indicating that there were significantly more large mussels (Size Class IV) found in the bottom layer than in the top layer.

The overall percentages of dead mussels in each layer were low, with slightly higher instances of dead individuals on the bottom, but no statistical difference between the two (Surface $\bar{x}$=0.60%;Bottom $\bar{x}$=3.00%:Paired T-test p=0.079, df=7).

Sampled water from within the colonies showed no significant differences in concentrations between the surface and bottom layers of the colonies (Fig. 9, Appendix Table C) for NO_3-N (Surface $\bar{x}$=0.301mg/L$\pm$0.0086, Bottom $\bar{x}$=0.293mg/L$\pm$0.012; Paired T-test, p=0.354,df=8), NH_3-N (Surface $\bar{x}$=0.136mg/L$\pm$0.075, Bottom $\bar{x}$=0.110mg/L$\pm$0.055; Paired T-test, p=0.085,df=8) and DIN (Surface $\bar{x}$=0.437mg/L$\pm$0.078, Bottom $\bar{x}$=0.402mg/L$\pm$0.062;Paired T-test, p=0.074.df=8).

The Effects of High Density and Various Flow Rates on Filtration Ability

Measured water flow speeds in the experimental recirculating flumes remained fairly consistent throughout each of the trials. At the highest ambient flow speed (~20cm/s), precise flow speed measurement was difficult due to the short length of the flume, so the measurement is an estimate.

Zebra mussels generally exhibited normal feeding behavior in each of the trials; the vast majority of observed individuals had open valves and

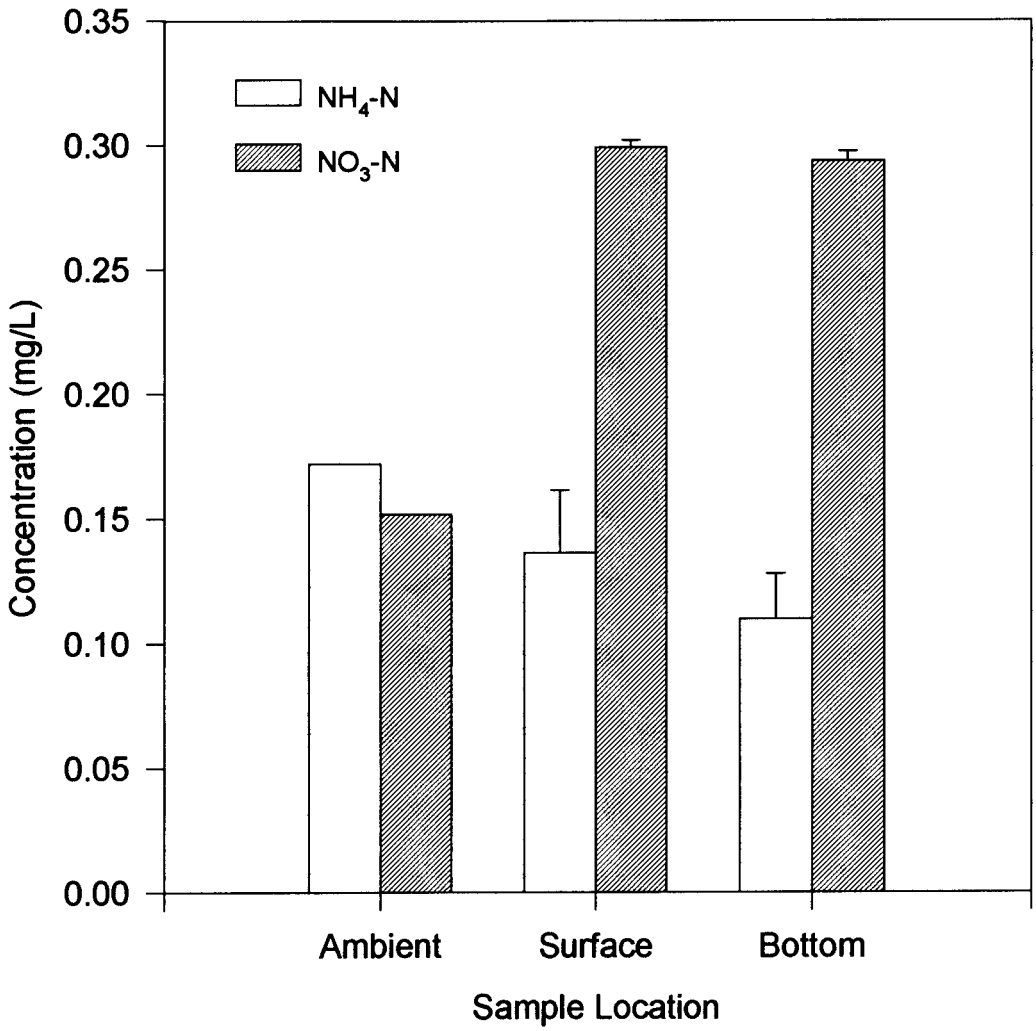

Figure 9. Mean concentration of dissolved NH_4-N and NO_3-N (+1s.e.) of ambient water and nine samples from the surface and bottom of a zebra mussel colony at Port of Indiana, Lake Michigan.

exposed siphons during each trial. A few individuals moved out of the colony chambers and onto the bottom or sides of the glass flume in some of the trials, but these represented <1% of the total colony population, and should not have affected the results. A few individuals located on the top of the colonies were swept out of the chambers by the flow in the ~20cm/s trials, but these were also <1% of the population. Otherwise, visual observations did not reveal any effect on behavior from differing flow speeds.

The radioactivity per gram of zebra mussel tissue indicated large differences in food consumption between the top and bottom layers of the colonies (Fig. 10; Appendix Table D). A 4-factor ANOVA was conducted on cpm/gram wet weight of sampled zebra mussel tissue, using flow speed, size class, day(trial)and location (top/bottom layer) as group variables. Ambient flow did not have an effect on colony filtration rate (Table 4). There was a significant interaction between size class and vertical position within the colonies. A post-hoc Tukey test revealed a significantly higher cpm/gram wet weight in the top layer than the bottom for each size class. There was also a significant increase in cpm/g wet weight with decreasing size in the top layer, but no significant difference among size classes in the bottom layer (Fig. 11).

The Effects of High Zebra Mussel Density on Vertical Migration and Mortality

The vast majority of the first group of labelled zebra mussels survived the painting procedure, with only 4 deaths out of more than three thousand

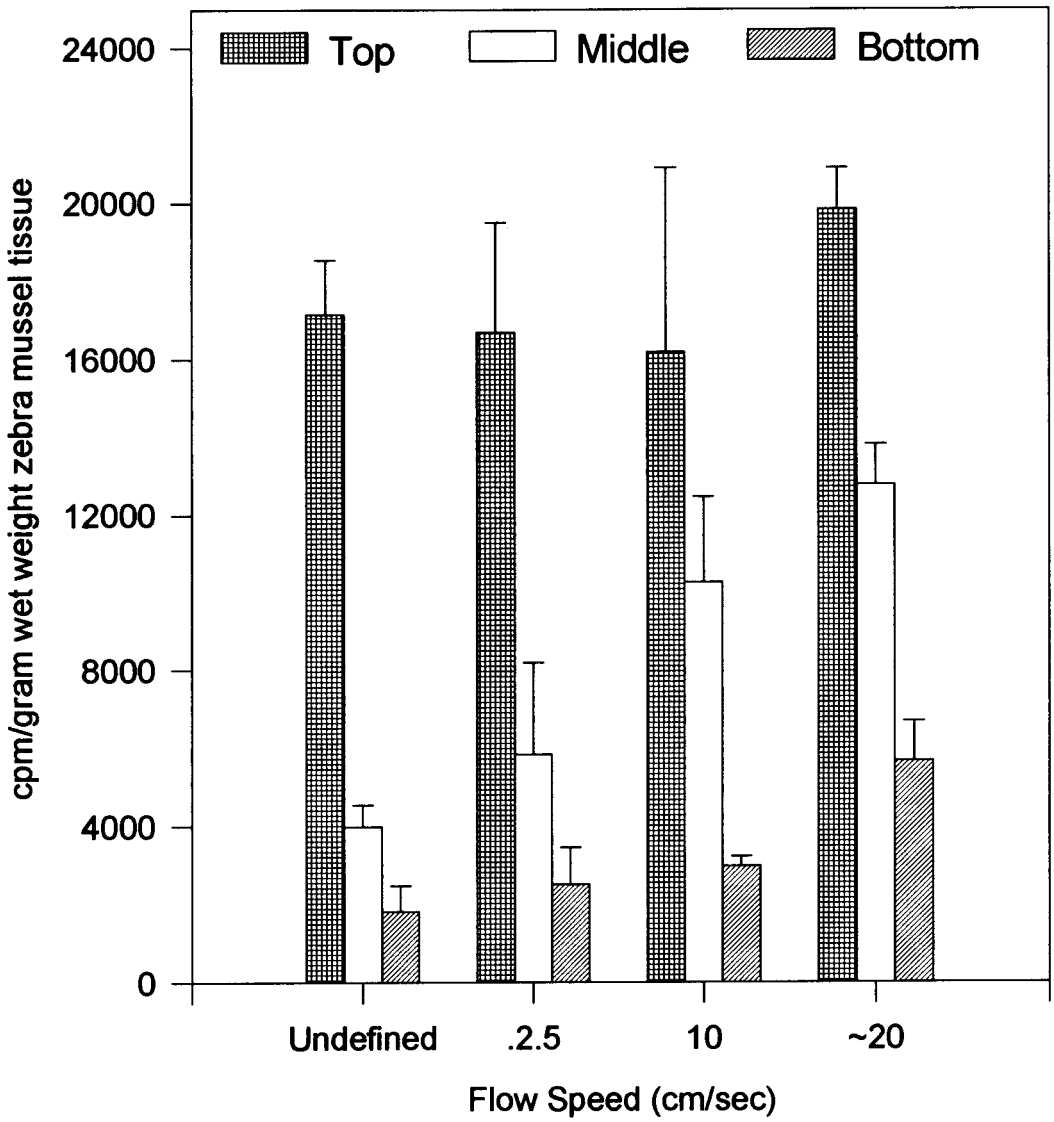

Figure 10. Feeding efficiency for three horizontal layers of zebra mussel colonies in four flow conditions. Feeding efficiency is expressed as mean radioactive count per minute/gram wet weight (+1s.e.) of zebra mussel samples from three trials.

Table 4. 4-factor ANOVA of cpm/gram wet weight of zebra mussel tissue
Fixed factors: Size Class(I,II,III), Position(Top/Bottom)
Random factors: Flow speed, Trial

Dependent Variable=cpm/gram tissue

Source	df	MS	F	p
Position	1	3.64×10^9	109.48	<0.001
Size Class	2	3.08×10^8	25.32	<0.001
Trial	2	4.90×10^7	7.14	0.009
Flow	3	4.61×10^7	1.27	>0.10
Flow x Position	3	3.43×10^6	0.02	>0.10
Position x Trial	2	3.32×10^7	4.84	0.029
Size Class x Position[a]	2	1.24×10^8	16.86	<0.025
Size Class x Trial	4	1.21×10^7	1.77	0.199
Flow x Size Class	6	2.46×10^7	0.56	>0.10
Flow x Trial	6	3.63×10^7	5.29	0.007
Size x Position x Trial	4	1.90×10^7	2.77	0.077
Size x Position x Flow	6	1.48×10^7	2.16	0.120
Position x Trial x Flow	6	4.39×10^7	6.40	0.003
Size x Trial x Flow	12	7.32×10^6	1.07	0.456
Error	12	6.86×10^6		

[a] Post-hoc Tukey test of Size Class x Position

TI	TII	TIII	BI	BII	BIII
(23,602)	(16,808)	(11,984)	(4,807)	(2,683)	(2,263)

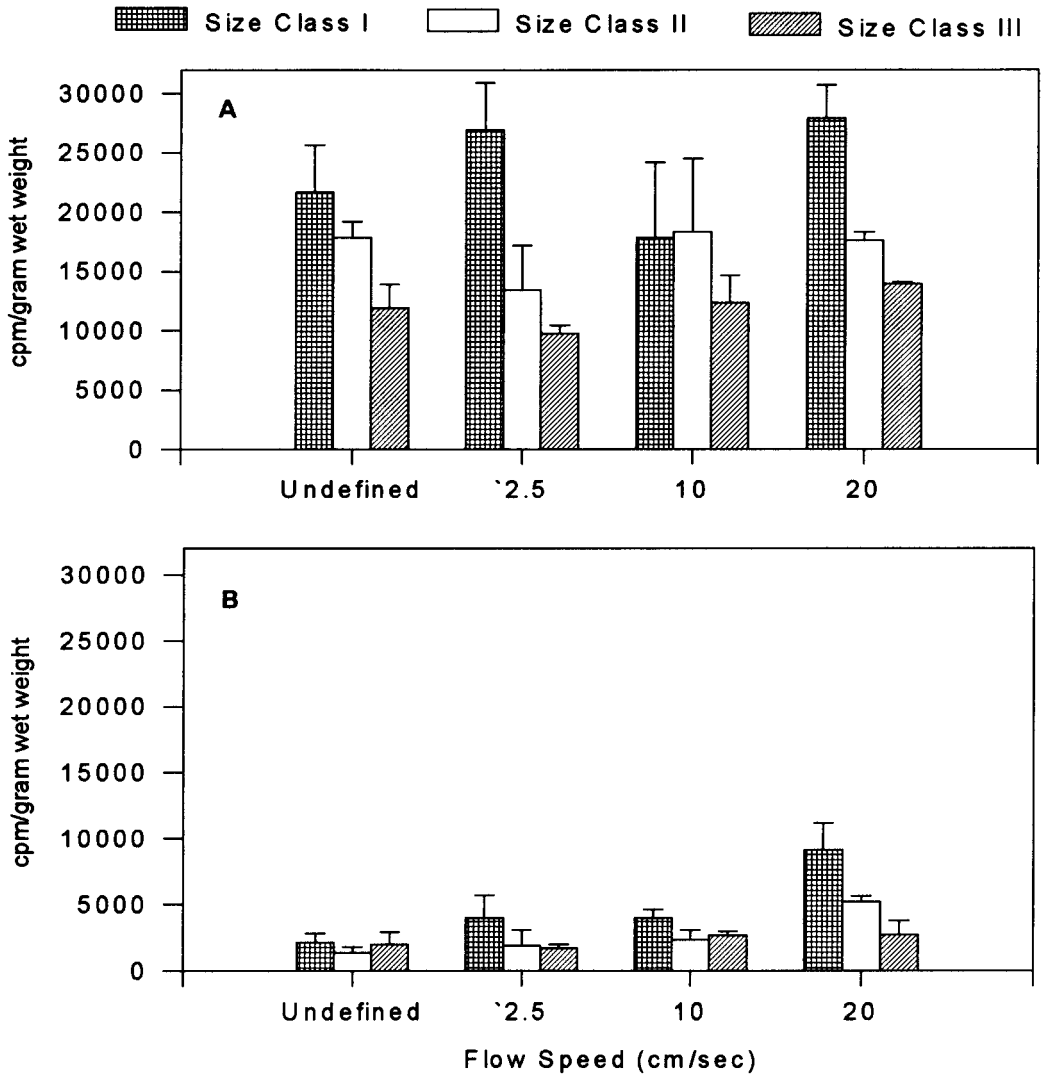

Figure 11. Algal feeding efficiency of three size classes of zebra mussels in four different flow conditions at the top layer of the colonies (A), and the bottom layer (B). Feeding efficiency is expressed as mean radioactive cpm/gram wet weight of zebra mussel sample (+1.s.e.) from three trials.

painted mussels.

Because water was pumped in directly from Lake Michigan, water temperature varied over time. The highest recorded temperature was 19.0°C, the lowest was 7.0°C. Mean recorded temperature was 13.5°C. Most of the experiments took place in conditions ranging from 11°C - 14°C. All experiments took place in temperature ranges that supported normal zebra mussel activity.

The 0 day control trials demonstrated the efficacy of the zebra mussel placement and sampling method. The majority of mussels sampled were still in the layer in which they had been placed. A mean of 93.0% of top labelled mussels, 88.6% of middle labelled mussels, and 99.6% of bottom labelled mussels were found in their respective layers. Of the mussels not found in their proper layer, 89.4% were found in a layer below. Most of this movement is probably due to the settling of individual mussels within the colony due to handling during the procedure.

The majority of sampled mussels in the 0 day trials were found to be alive. Dead mussels represented only 1.8% of those sampled. Dead mussels were found in roughly equal numbers in each of the levels. Vertical movement was rare, with an average of <0.4% of the mussels moving up from the bottom, and slightly more downward settling from the top layer (8.1%), most of which was by the smaller mussels (size class I, II).

Results from the time trials showed three major trends in mortality rate

in the zebra mussel chamber colonies: (1) overall mortality rates in the chamber colonies tended to increase over time, (2) overall mortality rates in the chamber colonies tended to increase with depth into the colony, and (3) mortality rates were not related to zebra mussel size class.

Mortality rates in the topmost layer of the chamber colonies remained very low in all of the time trials, ranging from 0-3.2% (Fig. 12). Mortality rates in the middle layer of the chamber colonies varied more with time than in the top layer. Rates were low in the 1 and 7 day trials, $\bar{x}$=1.27% and $\bar{x}$=0.84% respectively, and were comparable to those in the top layer, but increased slightly to 7.81% at 30 days (Fig. 13). Mortality rates increased the most over time, and were the highest overall, in the bottom layer. Overall mortality was fairly low in the 1 day trials (3.54%), but increased to 21.0% in the 7 day trials, and to 55.6% in the 30 day trials (Fig. 14).

A 4-factor analysis of covariance on mortality was conducted using size class, layer and trial# as a group variables and time as a covariate (Table 5). Zebra mussels on the bottom layer of the colonies experienced significantly higher mortality rates than those at the top, and there was a significant increase in mortality rate over time (30 day mortality, bottom $\bar{x}$=55.6%; top $\bar{x}$=0.39%; Fig. 14). Size class did not significantly affect mortality rates.

There were four trends in the vertical migration of zebra mussels in the chamber colonies: (1) There was a strong trend in upward migration by individuals in the bottom and middle layers of the chamber colonies, (2) there

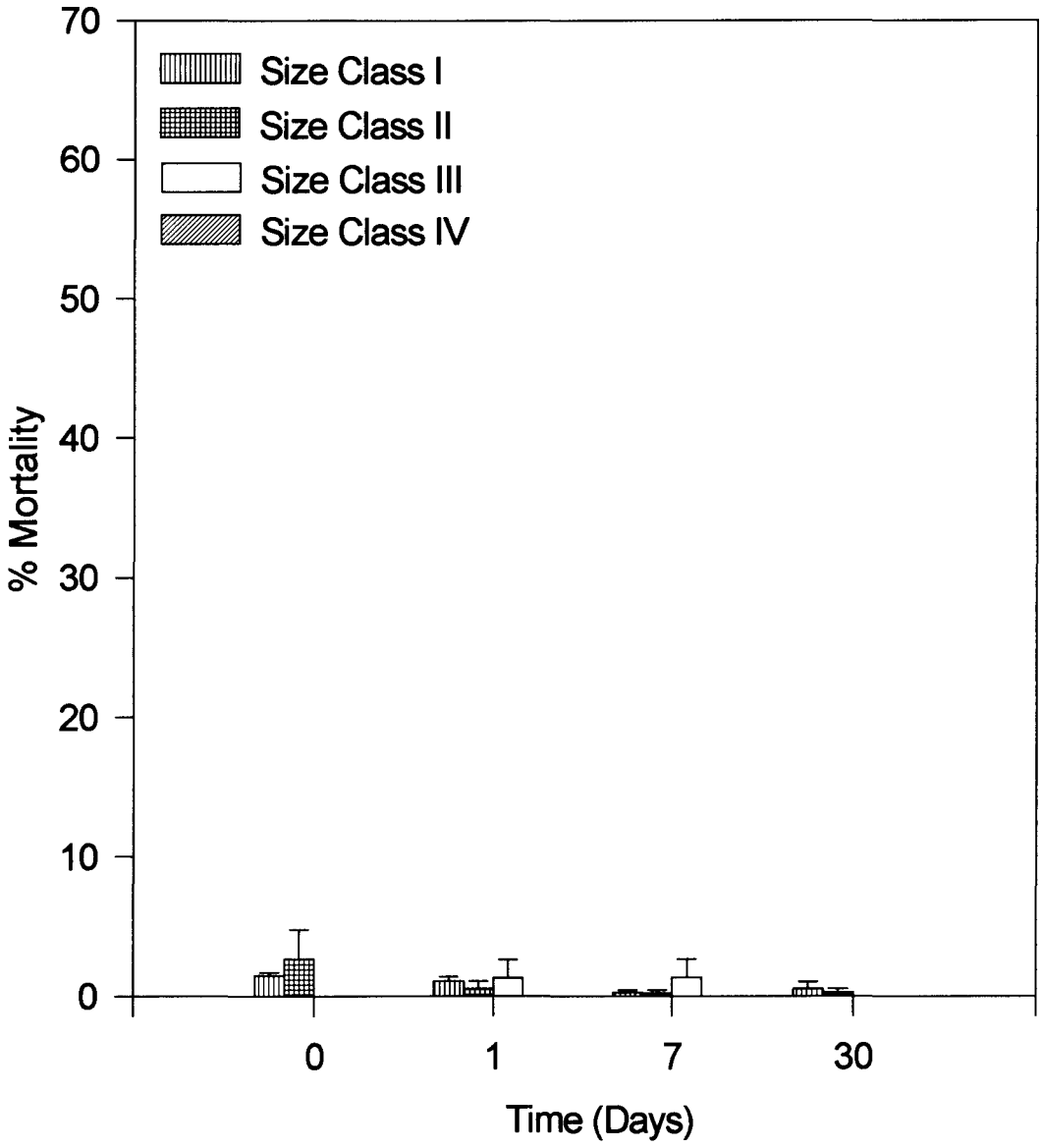

Figure 12. Mortality rates of four size classes of zebra mussels in four time trial periods in the top layer of the chamber colonies (+1s.e.). Size Class I (<6mm), Size Class II (6-13mm), Size Class III (13-20mm), Size Class IV (>20mm).

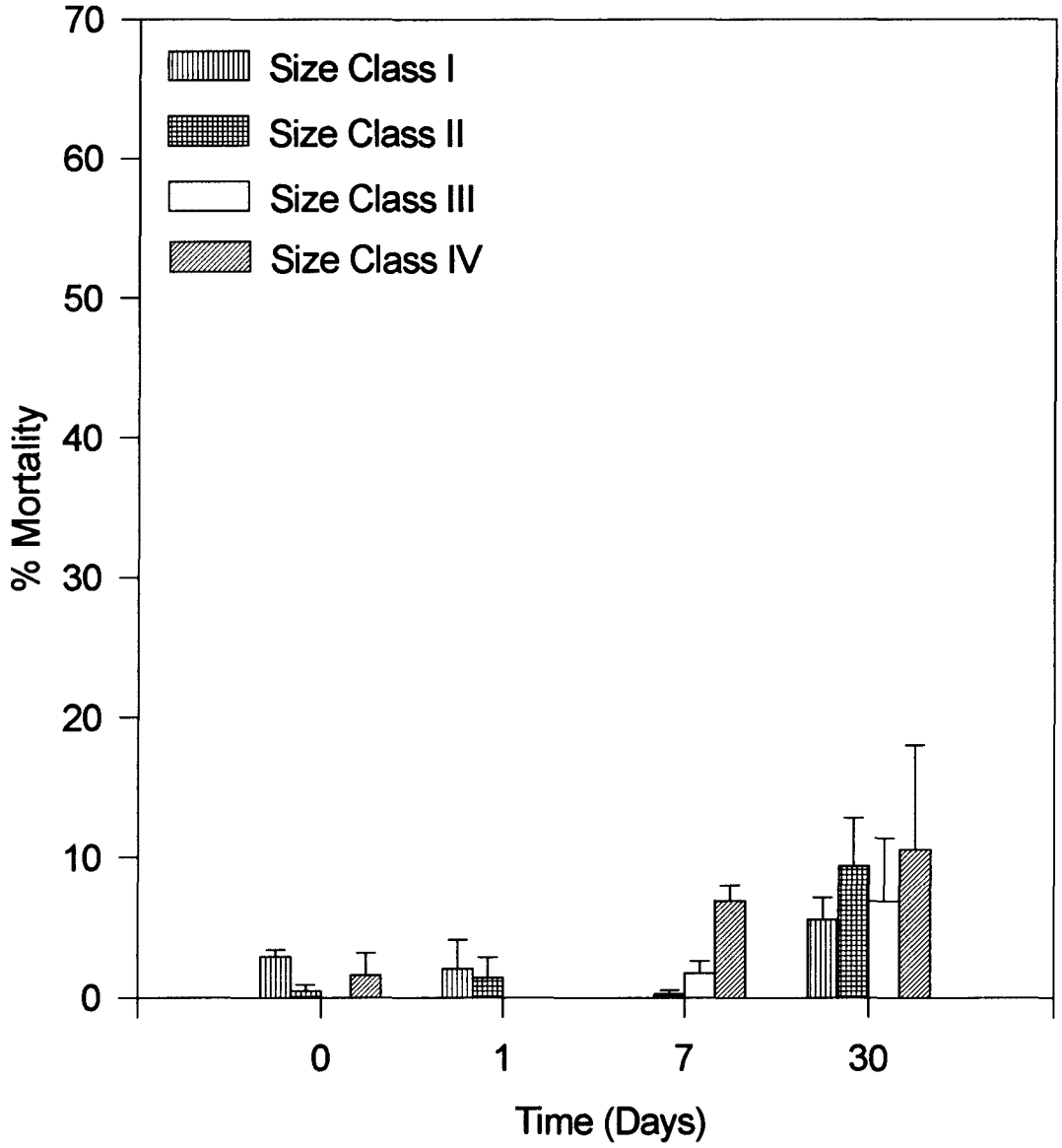

Figure 13. Mortality rates of four size classes of zebra mussels in four time trial periods in the middle layer of the chamber colonies. Size Class I <6mm, Size Class II 6-13mm, Size Class III 13-20mm, Size Class IV >20mm.

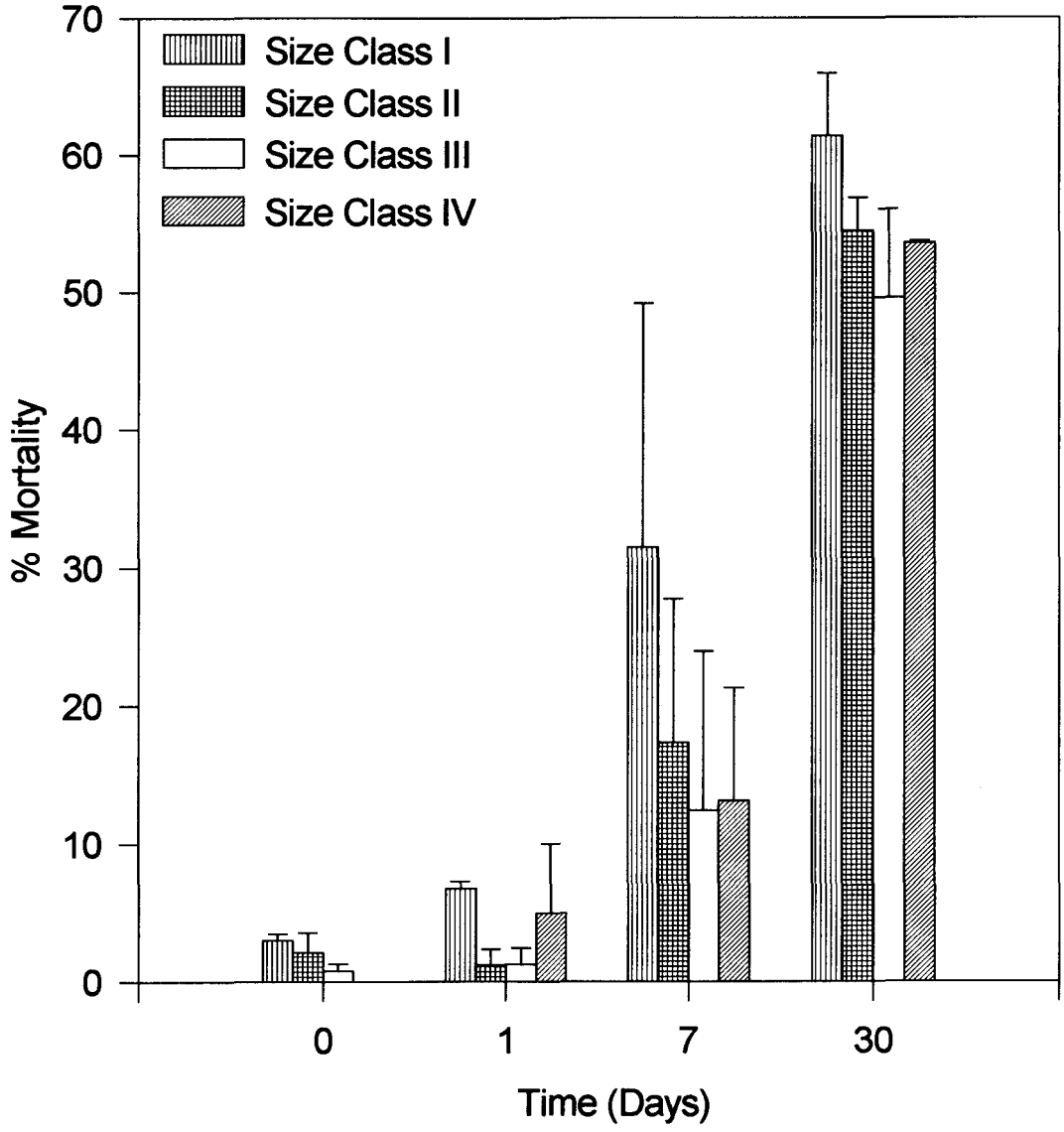

Figure 14. Mortality rates of four size classes of zebra mussels in four time trial periods in the bottom layer of the chamber colonies. Size Class I <6m, Size Class II 6-13mm, Size Class III 13-20mm, Size Class IV >20mm.

Table 5. 4-factor ANCOVA of zebra mussel mortality rate in the chamber colonies
Group variables: Size class, Layer, Trial#
Covariate: Time

Dependent Variable=Mortality

Source	df	MS	F	p
Trial#	2	156.429	0.973	0.383
Layer	1	6633.798	11.933	<0.001
Size Class	3	144.090	8.856	>0.05
Time	1	11054.545	68.794	<0.001
Trial x Layer	2	555.904	3.459	0.037
Trial x Size	6	16.269	0 .101	0.996
Layer x Size	3	61.376	3.85	>0.05
Layer x Size x Trial	6	15.933	0.099	0.996
Error	90	160.690		

was very little reciprocal downward migration by mussels in the top layer of the chamber colonies, (3) upward migration increased over time, and (4) upward migration rate was inversely related to individual size.

Upward migration rates (regardless of size) by zebra mussels in the bottom layer ranged from 11.36% in the 1 day trials to 48.19% in the 30 day trials. Downward migration from the top layer was less common, and more consistent over time, 9.45% at 1 day, 9.15% at 7 days, and 14.34% at 30 days. A 4-factor ANCOVA was conducted on the total rate of migration in the top and bottom layers of the colonies, with size class, layer and trial# as a group variables, and time as a covariate (Table 6). Smaller zebra mussels (Size class I, II) migrated significantly more than larger mussels (Fig. 15). Size class I mussels exhibited upward migration rates as high as 85.0% in the 30 day trials; size class II exhibited upward migration rates as high as 63.4%. Larger mussels did not exhibit much upward migration. Size class III had a maximum migration rate of 22.6% in the 30 day trials, and size class IV exhibited no upward migration. There was a significant increase in migration over time as well. Migration was generally upwards, with little downward migration from the top layer.

A 3-factor ANCOVA was performed on total migration rates in the middle layer of the colonies, with size class and trial# as group variables and time as a covariate. These data were analyzed separately because mussels in this layer could migrate both upwards and downwards. Migration in the middle

Table 6. 4-factor ANCOVA of migration rate in the top and bottom layers of the chamber colonies
Group variables: Size class, Layer, Trial#
Covariate: Time

Dependent Variable=Total Migration

Source	df	MS	F	p
Trial#	2	163.15	1.24	0.297
Size Class[a]	3	1439.54	58.45	<0.001
Layer	1	219.60	2.98	>0.05
Time	1	7089.22	53.66	<0.001
Trial x Layer	2	73.68	0.56	0.575
Trial x Size	6	24.63	0.19	0.980
Layer x Size	3	482.88	4.09	>0.05
Layerx Size xTrial	6	118.10	0.89	0.504
Error	71	132.10		

[a] Post-hoc Tukey test of Size Class

SCI(26.762) SCII(22.713) SCIII(13.476) SCIV(8.168)

3-factor ANCOVA of migration rate in the middle layer of the chamber colonies
Group variables: Size class, Trial#
Covariate: Time

Dependent Variable=Total Migration

Source	df	MS	F	p
Trial#	2	373.07	1.50	0.236
Size Class[a]	3	3069.26	55.41	<0.001
Time	1	1751.36	7.06	<0.001
Trial x Size	6	55.39	0.22	0.967
Error	35	248.19		

[a] Post-hoc Tukey test of Size Class

SCI(49.813) SCII(44.132) SCIII(28.315) SCIV(10.808)

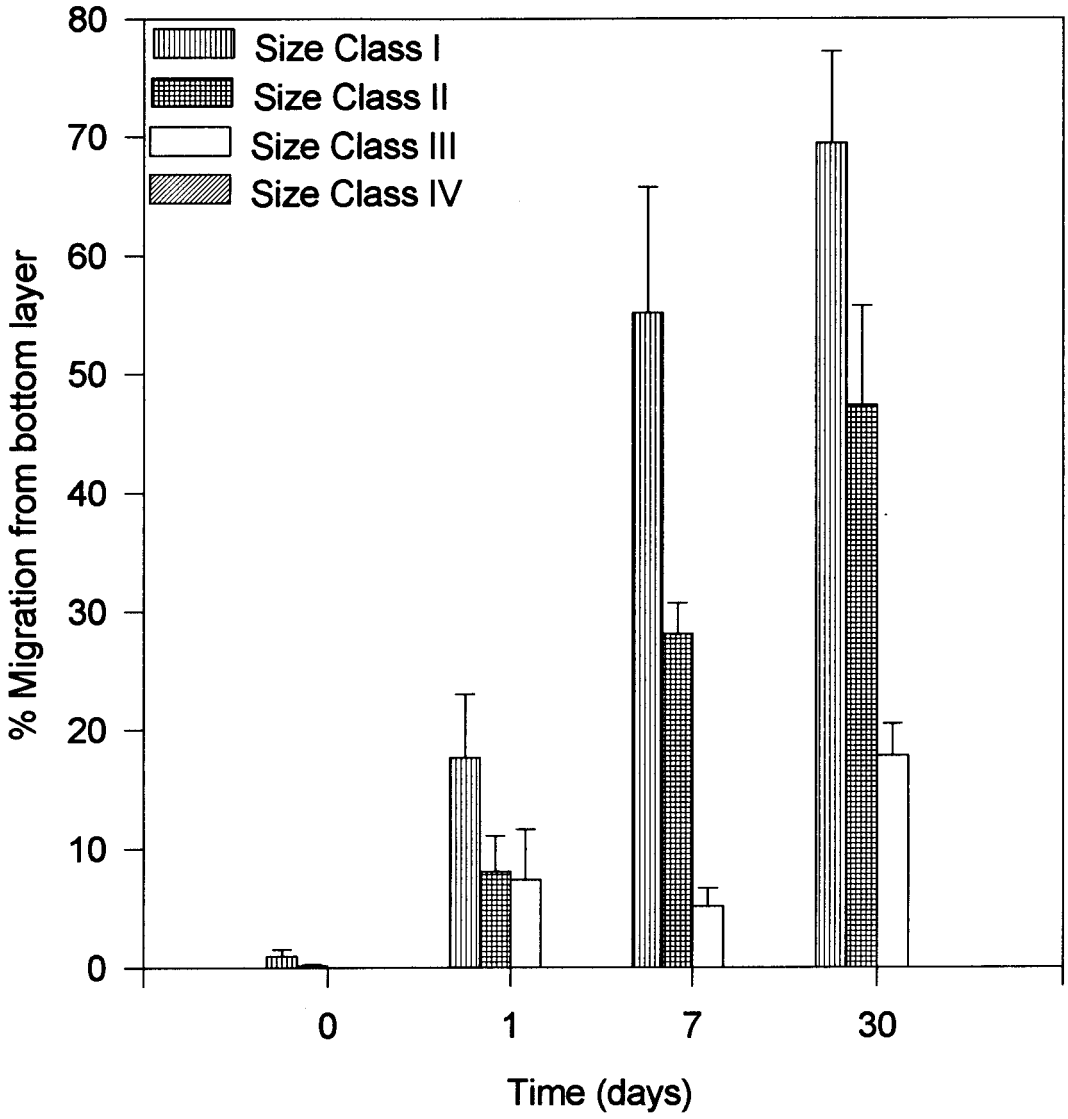

Figure 15. Rates of migration from the bottom layer of the chamber colonies for four size classes of zebra mussels in four time trial periods. Size Class I <6mm, Size Class II 6-13mm, Size Class III 13-20mm, Size Class IV >20mm.

layer tended to be upwards (64.58%) with little downward migration (6.97%) at 30 days. There was a significant difference in migration among size classes and over time, as in the top and bottom layers. In both cases, there was a significant increase in migration rate between each of descending size classes except Size class I and Size class II.

CHAPTER V

DISCUSSION

Interstitial Water Quality

The differences in dissolved inorganic nitrogen and dissolved oxygen (D.O.) concentrations between ambient water and the different locations within the chamber colonies indicate the presence of an overall water quality gradient. This gradient is probably derived from the combined metabolic activities of the individual zebra mussels within the colonies. Actively filtering mussels are consuming oxygen and excreting waste in the form of dissolved NH_4-N. A gradient would be formed when the rates of D.O. depletion and NH_4-N accumulation are greater than can be compensated for by diffusion or with exchange of ambient water along the surface of the colony. The water quality gradients in the colonies were vertical because the colony surface was on the horizontal plane. Although no gradients were seen in the field, vertically aligned colonies (e.g. on the sides of rocks) would have water quality gradients developing horizontally, away from the colony surface.

The steepness of a water quality gradient within a colony should be dependant on several factors. The density and size of a zebra mussel colony should affect water quality gradients. A greater number of metabolizing

individuals should contribute more NH_4-N and remove more D.O. than smaller, less dense colonies. A greater density of individuals should reduce the amount of interstitial space within a colony and interfere with the influx of ambient water. Larger, thicker colonies should have a greater percentage of individuals distal to the colony-water column interface, and would have potential for steeper gradients to develop. Similar trends in water quality gradients relative to colony thickness are seen on a much smaller scale in algal mats (Jorgensen et al., 1983; Burkholder et al., 1990). These factors would cause large, dense colonies to have steeper water quality gradients than smaller, less dense colonies under similar ambient conditions.

Temperature also has an effect on water quality gradients because zebra mussels filter water and metabolize at a faster rate in higher temperatures (Morton, 1971b). Quigley et al. (1993) showed that D.O. consumption and nitrogen excretion rates increased with temperature in individuals taken from Lake St. Clair.

Flow, in the form of both large scale ambient water movements and from the small scale siphonal currents of the mussels themselves, should also affect water quality gradients. At higher ambient flow rates, the rate of exchange of interstitial and ambient water would increase, reducing water quality gradients within the colony. Steeper gradients would occur in conditions with little or no ambient flow.

In low ambient flow rates, local interstitial water movements would be

dominated by the siphonal currents of filtering individuals. Zebra mussels do not orient themselves along their plane of substrate in response to flow or other factors (pers. observ.). In a large colony, the majority of individual mussels would be attached to other mussels, so both their horizontal and vertical orientation would be indeterminate. Therefore, it is unlikely that the siphonal currents of a large number of individuals would have an additive effect. Rather, they would probably tend to cancel each other out, causing only a recirculation of interstitial water. Ambient flow would most determine the net rate of water circulation at different depths within a large colony.

The factors of colony size and density, temperature, and ambient flow rate could conceivably combine to create areas of severely depleted D.O. within the colony, in some cases causing areas to become anoxic. Under anoxic conditions, excreted NH_4-N would not be converted into nitrate (a much less toxic form of nitrogen) and would accumulate to toxic levels. Zebra mussels have been demonstrated to be fairly intolerant of reduced D.O. levels (Stanczykowska, 1977). Levels of 2mg/l NH_4-N cause severe stress in zebra mussels, and levels <m/l cause 90-100% mortality (Nichols, 1993). The combination of low D.O. levels and elevated NH_4-N levels would create conditions extremely toxic to zebra mussels, as was demonstrated in the migration and mortality experiments under similar conditions.

In Situ Sampling

The absence of a dissolved nitrogen gradient in the *In situ* survey does not necessarily contradict the trends mentioned above, and is reasonable when viewed under the context of the history of a long term, stable population. A strong water quality gradient in a natural zebra mussel colony would indicate that the local population was in a temporary state of disequilibrium and would be too large to be supported under local conditions. If such a gradient were established, mortality might occur, reducing the population size. When the population reduces to a locally stable level, the gradient would fade. Therefore, these gradients could be the mechanism that limits population size in a local area.

Because individuals at the base of a colony would exist in poorer conditions than those near the surface, they would experience greater mortality rates. It is unclear, though, if the data taken from the cores support this trend. Although there was only a slight difference in the proportion of dead individuals between top to bottom, it is important to remember that the cores were taken from a vertical substrate. Byssal attachments are not directly connected to the valves of the zebra mussel shell (Eckroat, 1993). When the soft tissues of a mussel decay after death, its own byssal attachments no longer hold the shell in place. Unlike on a horizontal substrate, where gravity could keep unattached shells in place, on a vertical substrate unattached shells could fall out of the colony. In this case, the number of empty shells present might be an

underestimation of true mortality rates.

The size class distributions between the top and bottom portions of the colony cores were different. A greater portion of small mussels (size classes I, II) were found on the top relative to the bottom, while a greater portion of the largest mussels (size class IV) were found on the bottom. This difference could be due to age differences in the layers. If zebra mussel veligers settle on top of one another (Sprung, 1993), individuals on the bottom of a colony would be older and presumably larger. Younger, smaller individuals would be on the surface of the colony.

Another reason for the distribution of sizes might be due to migration by younger, smaller mussels, which are more likely to migrate than larger zebra mussels (Cawein, 1993). In this case, with a vertical substrate, it is unclear what kind of settlement patterns might be occurring. Based on the migration experiments in part IV, it is possible that the vertical stratification of size classes in these zebra mussel colonies is due to the active migration of smaller mussels rather than the sequential layering of different mussel cohorts.

One interesting phenomenon observed in the zebra mussel colonies at the Port of Indiana was their patchy, irregular depth. The surface of the colonies contained many raised patches, or "hummocks" of mussels. A similar trend was observed in large colonies of the blue mussel (*Mytilus edulis*) in the St. Lawrence estuary by Fréchette et al. (1989). Mussels on the top of these hummocks might have an advantage in feeding because of their increased

height above the substrate, which could expose them to greater food concentrations. It might also benefit the colony as a whole because the irregular surface provided by the hummocks would increase turbulent mixing, increasing the flux of food to the mussels downstream (Fréchette et al., 1989).

Zebra Mussel Filtration Rates

Individual mussels on the surface of the chamber colonies in the filtration experiments consumed more food than those on the bottom. Previous studies with individual zebra mussels have determined that food consumption rate is directly related to food particle density (Sprung & Rose, 1988). If that relationship is assumed here, then a gradient of food particle density existed within the interstitial water of the colonies.

Differences in food consumption rates by individuals in different locations within large colonies have been observed in other benthic filter feeders. In aggregations of the barnacle, *Balanus amphitrite*, upstream individuals capture significantly more food particles than downstream individuals (Pullen & LaBarbera, 1991). Other studies using growth rates as an indicator of feeding success found significantly greater growth rates in upstream individuals than downstream in the marine bryozoan, *Electra pilosa* (Okamura, 1992). Individuals in upstream locations of colonies of the blue mussel, *Mytilus edulis*, have higher growth rates than downstream individuals (Fréchette et al., 1992), and have greater growth rates on the edges of

colonies than in the middle (Okamura, 1986).

In these studies, food consumption and growth rates are related to an individual's position relative to the source of food particles. Individuals with lower food consumption and/or growth are exposed to food only after it has passed through other areas of the colony. The filtering actions of local members of the colony would reduce food density and quality, limiting their uptake and growth rates. Zebra mussels on the bottom of the experimental chamber colonies only received food after it had passed through the layers above, while zebra mussels on the surface received food directly from ambient flows. The same trend would be true for individuals with upstream-downstream, or exterior-interior relationships.

A major difference between the experimental zebra mussel chamber colonies and other benthic filter feeding colonies is that there was a vertical rather than horizontal food density gradient. Although it is probable that a horizontal gradient would also exist in a large zebra mussel colony, the vertical food density gradient would be much steeper, and would play a more important role in determining individual food consumption and growth rates than horizontal position within the colony.

Flow plays a major role in determining food consumption for different areas of colonies. In the recirculating flumes, increased flow speeds heightened food consumption in the middle and bottom layers of the chamber colonies. At higher flow speeds, turbulent mixing near the rough surface of the

colony would increase interstitial food densities. It would also improve water quality there, enhancing metabolic and pumping rates. This would increase the feeding rates of individuals in the lower portions of the colony.

Other studies on bivalve filtration have shown a negative correlation between flow speed and food consumption, probably due to an inhibitory effect on siphonal currents (Wildish et al., 1987). Cole et al. (1992) noticed that filtration rates of the clam, *Potamocorbula amurensis*, decreased with higher flow speed in recirculating flumes. Wildish et al. (1987) determined that growth by individuals of the giant scallop, *Placopectin magellanicus*, was inhibited at flow speeds of >10-20cm/s. Eckman et al. (1989) found similar trends at even lower speeds for the bay scallop, *Argopecten irradians concentricus*. In flume studies with the blue mussel, *M. edulis*, Wildish & Miyares (1990) found that filtration decreased with increasing flow speed, and was inhibited at >25cm/s. None of these studies, however, has compared food consumption in different areas of the same colony in different flows.

Another aspect of flow that affects filtration is the role of boundary layers. Many studies involving filtration by bivalves and other organisms have focused on the effects of the benthic boundary layer (Wildish & Kristmanson, 1984; Fréchette & Bourget, 1985; Fréchette et al. 1989; Butman et al. 1994), specifically on its role in food limitation. The physical properties of the benthic boundary layer as they relate to the study of benthic organisms have been incorporated into several flume designs as well (Muschenheim et al. 1986;

Nowell & Jumars, 1987).

The design used in this study did not incorporate stable benthic boundary layers, or otherwise try to quantify local flow, for several reasons. Because radioactive tracers were used, any flume used had to be recirculating, with a minimal volume to reduce radioactive waste. The establishment of a stable benthic boundary layer requires a prohibitively long flume design. Another consideration was the difference in substrate habitat. Unlike many marine bivalves, which inhabit soft and flat, sandy or muddy substrates, zebra mussels often colonize hard, highly irregular surfaces. These hard surfaces, whether they are large rocks or cobble, would break up stable benthic boundary flows, creating a more turbulent fluid environment. In such cases, the benthic boundary layer is probably not as relevant to filtration as in more flattened substrates. Because flows around and within zebra mussel colonies are dominated by turbulence, effectively quantifying them is extremely difficult.

The trend towards higher filtration/wet weight of zebra mussel in smaller individuals was unexpected. One possible explanation is the probable increase in gill/body mass ratio for smaller mussels compared to larger ones. A greater relative gill surface would allow mussels to filter particles more effectively. Another possibility is that the results are an artifact of the experimental design. Although *Chlamydomonas* is a free swimming algae, it is possible that some settlement could have occurred during the experiments, especially in the slower flow rates. Smaller zebra mussels have a higher surface area/wet

weight than large zebra mussels, which would allow higher algal settlement rates on the shell surfaces. A series of rinses with a sample of experimental mussels indicated that only relatively small amounts of radioactivity were present on the surface of the shell valves, not enough to account for the differences between size classes. Also, algal settlement would have been lower in the faster ambient flow speeds, and this trend was not seen in the data.

With the large differences in feeding rates in the three horizontal layers of the colonies in these experiments, individual food consumption efficiency becomes a factor of location. Zebra mussels at or near the surface of large colonies would have an advantage over those closer to the base. Because zebra mussels are mobile, and are capable of abandoning their byssal apparatus, competition could occur for space as a factor of food availability.

Similar situations have been demonstrated in other epifaunal benthic organisms. Buss (1979) described the competitive interactions of two infaunal marine bryozoans, *Onychocella alula*, and *Antropora tincta*, including competition for food and competition for space through overgrowth. In this case, higher food uptake would increase growth, coupling the two. The interdependence of space and food limitation was seen in studies of *M. edulis* colonies as well (Fréchette et al. 1992). In this case, growth was inhibited both through competitive interactions for food, and through interference from the crowding of other individuals. In these cases, competition for space takes place

through the settlement of new individuals, either on exposed substrate, or on top of other organisms. The role of individual migration and resettlement was not examined.

Similar relationships should occur in zebra mussels, with the added dimension of depth within a colony playing a role in competitive interactions. In the context of competitive interactions, food consumption by individuals at the bottom of the experimental colonies was inhibited by the presence of the mussels above them. Okamura (1986) noted that zebra mussels in large clumps grew more slowly than isolated individuals or those in small clumps. This was likely due to similar inhibitory competitive interaction. Because higher densities inhibit feeding efficiency, a given number of zebra mussels within a colony will not consume as much food as an equal number of separated individuals.

This trend towards lower feeding efficiency in higher colony densities has ramifications on the validity of current models and predictions on the effects of the feeding of entire zebra mussel populations. Current predictions of zebra mussel filtering impacts (MacIsaac et al. 1992; Bunt et al. 1992) are based on laboratory studies of the pumping rates of individual zebra mussels, or are extrapolated from the results of other laboratory studies on the filtration of individual zebra mussels (Kryger & Riisgard, 1988). Further, they assume a homogenous mixing of food particles within the water column, a condition that is extremely unlikely if not impossible. Because of the competitive inhibition of

food consumption efficiency, most of the individuals in large colonies of zebra mussels will not filter as effectively as isolated individuals. Therefore, any prediction about the filtering impacts of entire zebra mussel populations that is extrapolated from studies on isolated individuals would be highly overestimated. Actual assessments of phytoplankton populations support the likelihood that these are overestimations. Bunt et al. (1992) predicted that zebra mussel populations in western Lake Erie could theoretically filter 39-96% of the water column daily. *In situ* grazing experiments by Wu & Culver (1991) have shown that *Daphnia* grazing, rather than zebra mussel filtration, controlled phytoplankton populations in Lake Erie. Models that incorporate the effects of density, competition and localized food depletion would more accurately predict the impacts of the zebra mussel on phytoplankton populations.

Migration and Mortality

It has been established that zebra mussels are capable of abandoning their previously secreted byssal apparatus. This is done at the base of the byssus, near the gland (Eckroat, 1993). The specific cues that cause byssal abandonment are not known, but it is possibly a response to one or more adverse environmental conditions. Which environmental conditions an individual zebra mussel can sense and respond to is also not known. Unattached individuals have been observed to respond to concentrations of chlorine, potassium, and ammonia. Dissolved chlorine and potassium ions

induce complete cessation of movement and clamping of shells, while dissolved ammonia induces an increase in movement rate (pers. observ.). Zebra mussels have also been observed to abandon their byssal apparatus in response to high concentrations of several non-oxidizing molluscicides (McMahon et al., 1993). These responses occurred just prior to the death, so it is unclear how significant they were. Abandonment responses to natural cues are even less well known.

In this study, all of the zebra mussels that were tracked started with no byssal attachments. However, most of the mussels that moved in the experiments did so after at least seven days in the colony. Because mussels are capable of laying down byssal attachments immediately upon introduction to a substrate, it is possible that a large number of the mussels that migrated did so after abandoning their byssal apparatus.

One of the most noticeable significant trends in the experiments was the much higher instance of vertical migration in small mussels (size class I, II), than in large ones (Size class III, IV). Larger zebra mussels have a lower tendency to move than smaller ones when not bysally attached to a substrate (Cawein, 1993). This trend has been observed in other mussel species as well. Bertness & Grosholz (1985) determined that smaller individuals of the ribbed mussel, *Geukensia demissa*, moved statistically more frequently than larger individuals. Larger zebra mussels have been observed moving, so it is likely that they are behaviorally 'unwilling', rather than unable, to move as isolated

individuals. In addition, because the surface area of a large zebra mussel is greater than smaller ones, larger zebra mussels are much more likely to have the byssal attachments of other mussels on their shells. These attachments would make it more difficult, if not impossible, for the larger zebra mussels to migrate within a colony. In a large, dense colony, the number of larger individuals in this condition is probably quite high. In natural aggregations of *G. demissa*, greater than 99% of individuals were at least partially attached to other mussels (Bertness & Grosholz, 1985). Larger zebra mussels would be less likely to be able to move in between the other zebra mussels around them. Within a densely packed colony, larger zebra mussels with wider bodies would be less likely to be able to fit into the interstitial spaces of the colony that would give them a path to another location. Larger zebra mussels could be expected to be capable of moving with more force because of their size. However, byssal threads are capable of withstanding very high shear forces (Ackerman, 1993), so it is likely that most of the larger zebra mussels in a dense colony are essentially 'cemented' in place, and are unable to effectively migrate at all. Smaller zebra mussels, with a greater behavioral 'willingness' to move, a much lower likelihood of being burdened with other byssal attachments, and a greater capability of moving within the interstitial spaces of a dense colony, would have a much greater ability to migrate.

The specific cause for the large vertical migrations seen in these experiments is not known. Although vertical interstitial water quality gradients

were established in similar colonies during the water quality experiments, it was not possible to examine both water quality and migration simultaneously. Zebra mussels might be negatively geotactic, moving up in response to gravitational cues. This is unlikely, however, as zebra mussels avoid areas exposed to strong light (Marsden, pers. comm.). Natural populations tend to have greater densities in the darker areas underneath or on the sides of substrates than in the brighter areas on the top (Kilgour & Mackie, 1993; Yankovich & Haffner, 1993). Light avoidance may act as a stronger impulse than possible gravitational cues. In the experimental colonies, vertically migrating mussels were moving into areas of greater light intensity. Therefore, whatever drove them there was stronger than their urge to avoid light.

Another factor that might mitigate the differences in zebra mussel population densities between horizontal and vertical substrates is the effect of sedimentation. Horizontally aligned colonies, both in the flume experiments and in the dive sites, were observed to have accumulated a layer of sediment within and on top of the colonies. This sediment would serve to block interstitial spaces within the colonies, restricting flow. Decaying organic matter within the sediment would contribute to the reduction of water quality as well. Vertically aligned colonies would not experience these effects.

It is much more likely that vertically migrating zebra mussels were responding to water quality gradients similar to those defined above. Migration increased after seven days, which corresponds with the time that higher levels

of mortality were first seen in the bottom layer of the colonies. If mortality was due to poor water quality, then migrating mussels did not tend to move until those conditions developed. Based on their observations of movement behavior in the ribbed mussel (*G. demissa*), Bertness & Grosholz (1985) suggested that juveniles were capable of actively selecting habitats that were favorable for growth and survival, and migrating to them. It is likely that similar forces are at least partly responsible for the migration patterns in these colonies.

The specific cause of mortality in the colonies is also difficult to determine. Whatever the cause, it was present only after a certain amount of time, and then only in the bottom layer of the colony. The results of the filtration experiments, indicating that food consumption decreases with depth into a colony, would apply here. Based on those results, it can be assumed that mussels in the bottom layer of the colony were consuming much less food than those in the top layer. However, zebra mussels can survive in lab conditions for more than four months without food (Nichols, 1993), so it is unlikely that starvation was the sole cause of mortality.

Poor water quality, in the form of depleted D.O. levels and elevated NH_4-N levels, is the most likely cause of mortality. Zebra mussels are fairly intolerant of low D.O. levels (Stanczykowska, 1977), but are also intolerant of NH_4-N. Levels of 2mg/l NH_4-N cause severe stress in zebra mussels, and levels >4m/l cause 90-100% mortality (Nichols, 1993). Because most NH_4-N

was converted to NO_3-N in similar colonies, and because NH_4-N levels do not often elevate until water becomes anoxic, it is likely that the cause of death of most of the mussels in this study was suffocation.

The results of the interstitial water quality experiments indicate that mortality due to poor water quality would occur farthest away from the surface of the colony. In each of the mortality experiments, this was the case. Mortality in the upper layers of the colonies only occurred in the presence of much higher mortality rates below.

With high mortality rates in one area of a colony, it is possible that a chain reaction could start. Dead mussels would begin to decay, causing local NH_4-N levels to rise. This in turn would cause more deaths, further increasing NH_4-N levels. In such instances, without the flow of water to relieve toxic conditions, mortality rates would be severe. Although this is common in poorly maintained laboratory tanks, it is probably less common in nature. When it does occur, mortality would begin in areas away from the surface of the colony, probably at the bottom. In such a case, any attachments connecting those mussels to the substrate would no longer hold. Although individual mussels on the surface of the colony would still be alive, their indirect attachment to the substrate would be lost. In conditions with unusually strong ambient currents (e.g. wave action from a storm), sloughing might occur, removing aggregations of living mussels and exposing substrate. Exposed surfaces would subsequently be recolonized by veliger settlement and adult

migration. This could be partially responsible for the patchy, irregular surface of some colonies. Zebra mussels sloughed off by the current might survive and reaggregate, and it is possible that round zebra mussel druses, often observed on sandy or muddy bottoms, originated as portions of hard substrate colonies.

In nature, most zebra mussel colonies do not achieve the densities that were used in these experiments. However, colonies exceeding these densities, especially in man-made conditions (e.g. intake pipes), do exist. Because of a greater supply of food and substrate, and a possible lack of predators, it is likely that zebra mussels will reach the maximum density that can be supported by local conditions shortly after their initial colonization. When those densities are exceeded, whether by over colonization of a local area, or by a change in local conditions that limits population size, the population would be reduced in much the same way as has been shown above, with mortality affecting individuals along a gradient of interstitial water quality away from the colony surface.

Zebra mussels in areas of poor water quality are at a disadvantage compared to those in areas of high water quality. Those mussels in poor quality areas that can migrate into a better area have an advantage over those that cannot. This competition for space, as a factor of water quality, makes dense zebra mussel colonies active, dynamic populations that change over time in response to changing ambient and interstitial conditions.

CHAPTER VI

CONCLUSIONS

Zebra mussels are most often encountered as individuals existing in large, dense colonies. The focus of this study was to examine the role of high colony densities on aspects of individual zebra mussel biology.

These experiments have shown that many of the impacts that zebra mussels have on their immediate environment are negative. Because of the additive effects of their metabolic activities, water quality gradients can develop in the interstitial waters of a zebra mussel colony, with the poorest water quality occurring in areas farthest from the colony surface. Levels of D.O. are reduced, and levels of NH_4-N and NO_3-N are increased, creating conditions that can be stressful or even fatal to them and any other organisms living there.

The filter feeding activity of the individuals within a colony also has a negative impact. The combined effects of filtration create gradients of food particle density, and can cause areas of food reduction or depletion, which would also occur farthest from the colony surface. As a result, individuals in those areas could eventually face starvation.

These gradients of water quality and food availability in the interstitial

waters of a colony are impacted by the flow of water. The rate of influx of ambient water to the interstitial spaces of a colony is dependent on the colony surface area and roughness, its density and thickness, and ambient flow. Increased flow would decrease interstitial gradients relative to slower flow.

The effect that these gradients have on zebra mussels within a colony is dependent primarily on individual location. Zebra mussels farther from the surface of a colony would experience poorer conditions than those near or at the surface. Therefore, it is likely that competition for water quality and resources would occur as a function of space, or location within a colony.

These experiments have shown that migration does occur within colonies. Individual zebra mussels migrate towards the surface of colonies, presumably to achieve a better position within the colony and escape the poorer conditions near the base. Individuals that are unwilling or unable to escape from those conditions suffer much higher mortality rates than those in the better conditions at the surface.

The combination of decreased mortality and increased food availability at the surface of zebra mussel colonies gives the individuals that live there a selective advantage over those that live near the base. This advantage would allow for competition to occur for space within a colony, a competition that would be won by those individuals that were capable of migration.

Zebra mussel colonies are complex, dynamic systems, formed by the many processes and interrelationships among the individuals therein. By

incorporating these factors into zebra mussel studies, a more accurate, complete picture of their biology and their effect on the environment can be seen.

APPENDIX

Table A. Interstitial Water Quality Data

Ammonia Concentration (mg/L)								
	Date: 10/12/1993	T=11c		10/15/93	T=11c			
Flow:	1cm/sec	1)	2)	3)	4)	Avg.	StDev.	
Sample	Ambient	0.14	0.12	0.038	0.039	0.084	0.0535	
Location:	top/upstream	0.515	0.022	0.035	0.033	0.151	0.2426	
	top/midstream	0.255	0.054	0.044	0.029	0.096	0.1068	
	top/downstream	0.085	0.135	0.036	0.034	0.073	0.0479	
	center/upstream	0.081	0.03	0.054	0.038	0.051	0.0225	
	center/midstream	0.091	0.052	0.045	0.037	0.056	0.024	
	center/downstream	0.17	0.065	0.063	0.034	0.083	0.0597	
	bottom/upstream	0.18	0.098	0.029	0.037	0.086	0.0698	
	bottom/midstream	0.465	0.055	0.055	0.041	0.154	0.2074	
	bottom/downstream	0.075	0.038	0.044	0.035	0.048	0.0184	
	Mean	0.213	0.061	0.045	0.03533	0.089	0.0888	
Nitrate Concentration (mg/L)								
	10/12/93			10/15/93				
Flow:	1cm/sec	1)	2)	3)	4)	Avg.	StDev.	
	Ambient	0.285	0.365	0.295	0.375	0.33	0.0465	
	top/upstream	0.35	0.45	0.33	0.43	0.39	0.0589	
	top/midstream	0.4	0.47	0.35	0.465	0.421	0.0572	
	top/downstream	0.4	0.44	0.355	0.435	0.408	0.0393	
	center/upstream	0.475	0.495	0.42	0.455	0.461	0.032	
	center/midstream	0.575	0.745	0.62	0.725	0.666	0.0819	
	center/downstream	0.505	0.52	0.44	0.615	0.52	0.0722	
	bottom/upstream	0.61	0.615	0.45	0.645	0.58	0.088	
	bottom/midstream	0.835	0.645	0.63	0.695	0.701	0.0934	
	bottom/downstream	0.54	0.57	0.515	0.675	0.575	0.0704	
	Mean	0.52111	0.55	0.45667	0.57111	0.525	0.0659	

Table A. (continued)

Combined DIN Concentration (mg/L)									
		T=11.5c			T=11.2c				
		10/12/95			10/15/95				
Flow:	1cm/sec	1)	2)	3)	4)	Avg.	StDev.		
Sample	Ambient	0.425	0.485	0.333	0.414	0.41	0.063		
Location:	top/upstream	0.865	0.472	0.365	0.463	0.54	0.221		
	top/midstream	0.655	0.524	0.394	0.494	0.52	0.108		
	top/downstream	0.485	0.575	0.391	0.469	0.48	0.075		
	center/upstream	0.556	0.525	0.474	0.493	0.51	0.036		
	center/midstream	0.666	0.797	0.665	0.762	0.72	0.067		
	center/downstream	0.675	0.585	0.503	0.649	0.6	0.077		
	bottom/upstream	0.79	0.713	0.479	0.682	0.67	0.133		
	bottom/midstream	1.3	0.7	0.685	0.736	0.86	0.297		
	bottom/downstream	0.615	0.608	0.559	0.71	0.62	0.063		
	Mean	0.73411	0.611	0.502	0.60644	0.61	0.12		
Dissolved Oxygen Concentration (mg/L)									
		10/12/95			10/15/95				
Flow:	1cm/sec	1)	2)	3)	4)	5)	6)	Avg.	StDev.
Sample	Ambient	10.5	10.5	10.5	10.8	10.8	10.8	10.7	0.164
Location:	top/upstream	11	10.4	10.6	10.8	10.7	10.7	10.7	0.2
	top/midstream	11	10.3	10.6	10.8	10.7	10.7	10.7	0.232
	top/downstream	11	10.4	10.4	10.8	10.7	10.7	10.7	0.234
	center/upstream	10.1	9.9	10.4	10.4	9.8	10.2	10.1	0.25
	center/midstream	10	9.9	10	10	9.3	10.1	9.88	0.293
	center/downstream	9.8	9.7	10	9.9	9.4	10.2	9.83	0.273
	bottom/upstream	8.9	10	9.6	9.8	9.8	10	9.68	0.412
	bottom/midstream	8.6	8.2	8.2	9	8.6	9.8	8.73	0.602
	bottom/downstream	9	8.9	9.3	9.4	8.6	9.8	9.17	0.423

Table B. Natural Zebra Mussel Colony Core Population Data

Zebra Mussel Core Data		(# of Zebra Mussels of each size class in each core)							
Taken: 8/11/94	Size Class:								
Location:	Core#:	I	% Total	II	% Total	III	% Total		
Top	1	69	36.9	69	36.9	37	19.79		
	2	51	22.27	121	52.84	50	21.83		
	3	59	34.5	72	42.11	39	22.81		
	4	68	30.09	95	42.04	55	24.34		
	5	14	9.52	61	41.5	65	44.22		
	6	57	23.65	150	62.24	24	9.96		
	7	45	19.4	141	60.78	42	18.1		
	8	175	44.76	171	43.73	41	10.49		
	Total	538	29.5	880	48.25	353	19.35		
		Size Class:							
Location:	Core#:	I	% Total	II	% Total	III	% Total		
Bottom	1	25	40.32	15	24.19	10	16.13		
	2	11	18.03	25	40.98	13	21.31		
	3	18	21.18	38	44.71	20	23.53		
	4	7	26.92	7	26.92	2	7.69		
	5	14	23.73	26	44.07	11	18.64		
	6	26	30.23	39	45.35	8	9.3		
	7	6	10.53	37	64.91	8	14.04		
	8	0	0	13	43.33	7	23.33		
	Total	107	22.96	200	42.92	79	16.95		
					# of Dead Zebra Mussels:				
Location	Core#	IV	% Total	Total	I	II	III	IV	Total
Top	1	12	6.42	187	0	0	0	1	1
	2	7	3.06	229	0	0	0	0	0
	3	1	0.58	171	0	0	0	0	0
	4	8	3.54	226	0	0	1	0	1
	5	7	4.76	147	0	0	0	0	0
	6	10	4.15	241	1	1	1	0	3
	7	4	1.72	232	0	0	1	1	2
	8	4	1.02	391	2	0	2	0	4
	Total	53	2.91	1824	3	1	5	2	11
Location:	Core#:	IV	% total	Total	I	II	III	IV	Total
Bottom	1	12	19.35	62	0	0	0	0	0
	2	12	19.67	61	0	0	0	4	4
	3	9	10.59	85	0	0	0	2	2
	4	10	38.46	26	0	1	0	0	1
	5	8	13.56	59	0	2	0	0	2
	6	13	15.12	86	0	0	0	1	1
	7	6	10.53	57	0	0	0	0	0
	8	10	33.33	30	0	1	1	2	4
	Total	80	17.17	466	0	4	1	9	14

Table C. Natural Zebra Mussel Colony Interstitial Water Quality Data

Interstitial Water Quality Data from a Natural Zebra Mussel Colony									
Samples taken by SCUBA at a stone breakwall, Port of Indiana, Lake Michigan									
Date: 8/11/94		Concentration in mg/L							
Location	Sample	Ammonia	Nitrate	Total N	Location	Sample	Ammonia	Nitrate	Total N
Bottom	1	1.32	0.285	1.605	Top	1	1.23	0.323	1.553
	2	0.204	0.295	0.499		2	0.319	0.305	0.624
	3	0.177	0.299	0.476		3	0.163	0.305	0.468
	4	0.075	0.293	0.368		4	0.123	0.3	0.423
	5	0.085	0.304	0.389		5	0.126	0.305	0.431
	6	0.065	0.29	0.355		6	0.09	0.286	0.376
	7	0.076	0.29	0.366		7	0.11	0.291	0.401
	8	0.066	0.272	0.338		8	0.083	0.312	0.395
	9	0.076	0.286	0.362		9	0.067	0.295	0.362
	10	0.162	0.313	0.475		10	0.146	0.291	0.437
	Ambient	0.152	0.172	0.324		Ambient	0.152	0.172	0.324
	Average*	0.10956	0.293	0.4023		Average*	0.13633	0.301	0.4376
	* The first ammonia sample at each location is an outlier								
	It was not included in data analysis								

Table D. Zebra Mussel Colony Filtration Data

	32P Filtration Experiment Data						
	Counts/gram wet weight zebra mussel tissue						
		Replicate #					
Flow Speed:	Site/Size	1	2	3		Avg.	St.Dev.
(cm/s) 0	T I	29430.04	19046.02	16619.15		21698.4	6804.86
	T II	20200.42	15560.04	17803.78		17854.7	2320.61
	T III	9548.262	10214.65	15899.74		11887.6	3490.6
	M	3329.084	3531.932	5114.547		3991.85	977.556
	B I	2980.393	2576.463	856.8518		2137.9	1127.66
	B II	1883.735	1734.828	453.5754		1357.38	786.25
	B III	3691.479	1853.885	399.4431		1981.6	1649.73
2.5	T I	21414.22	34654.4	24784.05		26950.9	6880.92
	T II	8461.111	20796.34	10973.72		13410.4	6518.63
	T III	9061.328	11109.64	9008.707		9726.56	1198.07
	M	3457.768	3508.269	10548.69		5838.24	4079.44
	B I	1441.18	3311.338	7245.309		3999.28	2962.59
	B II	805.0396	561.9258	4252.278		1873.08	2064.03
	B III	1214.575	2298.692	1545.969		1686.41	555.536
10	T I	27700.31	19790.76	6019.407		17836.8	10971.7
	T II	22182.75	26611.69	6241.751		18345.4	10713.4
	T III	13447.79	15732.96	7830.259		12337	4066.76
	M	12176.87	12738.62	5851.083		10255.5	3824.69
	B I	3807.81	5154.464	2945.522		3969.27	1113.29
	B II	1511.542	1699.794	3737.23		2316.19	1234.25
	B III	2163.747	3019.841	2853.091		2678.89	453.854
20	T I	33462.87	24221.72	26081.48		27922	4887.78
	T II	17955.11	16209.86	18704.7		17623.2	1280.1
	T III	14055.12	14179.15	13720.82		13985	237.067
	M	12744.59	10999.69	14574.67		12773	1787.66
	B I	9742.178	12332.23	5301.594		9125.33	3555.68
	B II	6116.659	4730.016	4716.489		5187.72	804.512
	B III	2072.435	4774.64	1273.151		2706.74	1834.9
	Blank 1		47.02				
	Blank 2		51.2				
	Blank 3		48.6				
	Blank 4		45.3				
	Blank 5		46.8				
	Blank 6		46				
	Average		47.01667				

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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

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